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Regional blood brain permeability-capillary surface area products and regional cerebral blood flows were significantly increased by subconvulsant and convulsant treatment with soman. These effects were dose-related and time-dependent. Some effects lasted as long as 1 week, even when subconvulsant doses were used. The pattern and intensity of effects were qualitatively and quantitatively different from those produced by pentylenetetrazol. Soman also produced marked neuropathology across wide brain regions. Combined anticonvulsant pretreatment with diazepam, HI-6 and atropine failed to prevent much of the long-term cerebrovascular damage and neuropathology produced by soman. Strategies for enhancing the neuroprotective actions of this pretreatment are described.

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Harold Goldman 6/13/91
PI - Signature DATE

Regional blood-brain barrier responses to central cholinergic activity

TABLE OF CONTENTS

Regional blood-brain barrier responses to central cholinergic activity

SUMMARY

The objectives of our research were twofold: 1) to determine the time-dependent changes in cerebrovascular functions (i.e., regional cerebral blood flow (rCBF), regional blood-brain barrier permeability (rPS), brain vascular space) following exposure to the anticholinesterase soman and the roles of those changes in producing damage to the central nervous system (CNS); and 2) to examine the effectiveness of two treatment strategies, one conventional, the other novel, in preventing the serious cerebrovascular consequences of such exposure. The non-organophosphate convulsant pentylenetetrazol (PTZ) was used as a reference compound, against which to compare microvascular and histopathological changes following convulsant and subconvulsant exposure to soman. This work represents the first comprehensive, quantitative analysis of time-dependent, regional cerebrovascular responses to subconvulsant and convulsant doses of an organophosphorus cholinesterase inhibitor, soman, and includes comparisons with responses to similar treatments with another type of convulsant, pentylenetetrazol.

APPROACH

The irreversible anticholinesterase soman produces toxic effects on the central nervous system, even when administered in a single subcutaneous injection (McDonough et al., 1986; Shih, 1982). These include tremor, convulsions and respiratory failure, with long-term behavioral changes in those animals which survive the initial insult. The toxic action of soman is probably due to the accumulation of acetylcholine (ACh) at central synapses leading to hyperactivity of the cholinergic system. Soman also causes lesions and degeneration throughout the nervous system (Honchar et al., 1983; McDonough et al., 1986), with the most extensive damage found in limbic system structures (i.e., piriform cortex, amygdala, entorhinal cortex and hippocampus). Since cholinergic agents are known to alter regional cerebrovascular permeability, and since muscarinic cholinergic receptors and cholinesterase activity are known to be associated with CNS vasculature, we have hypothesized that soman-induced pathology, and that produced by anticholinesterases in general, may be partly due to changes in cerebrovascular function.

Studies carried out in the late 1940's and 1950's indicated that seizures produced by soman and other anticholinesterases also were associated with increased blood-brain barrier permeability (Greig and Mayberry, 1951; Greig and Holland, 1949). However, these earlier studies gave conflicting results due, in part, to outdated methodologies. This earlier line of work was abandoned and largely forgotten. Recent work, employing essentially the same qualitative markers of permeability, including Evan's Blue (Carpentier et al., 1990), have produced only

limited results, which are at odds with our more detailed quantitative findings described below. The work reported here on soman-induced seizures, regional cerebral blood flow and regional cerebral permeability, employing more sensitive and quantitative methods, have been substantially revealing in this regard.

Initial experiments focused on characterizing seizures produced by pentylenetetrazol (PTZ) and on determining their effects on cerebrovascular function. Parenteral administration of PTZ produced, sequentially, myoclonus, clonic, and then tonic seizures. Seizures were monitored electrographically by chronically implanted epidural electrodes. Their time of onset and duration were dose-dependent. The mechanisms underlying PTZ seizures are only partially known, but may involve the GABA, noradrenergic and adenosine systems (Burley and Ferrendelli, 1984; Wasterlain, 1989). Specifically, PTZ blocks GABA receptor function, probably by blocking the benzodiazepine site linked to the $GABA_a$ receptor (Wasterlain, 1989). These PTZ seizures are of interest because they are well characterized and not mediated by the cholinergic system. Therefore, they provide an excellent seizure model for comparison with soman-induced seizures.

In subsequent studies, the effects of PTZ seizures on mean arterial blood pressure (MABP), cardiac outputs, hematocrit, rPS, rCBF, and brain vascular space (BVS) were characterized. The cerebral microvasculature (and its blood-brain barriers) occupies a central position in the regulation of the internal environment of the brain and spinal cord. Short- and long-term effects on both the structure and function of the central nervous system would be likely to follow traumatic events (i.e., convulsive seizures) to the microvasculature. Blood flow and permeability changes have been reported to follow drug-induced seizures (Greig and Hellmann, 1983; Ingvar et al., 1984; Ruth, 1984; Ziylan and Ates, 1989; Johansson and Linder, 1978). However, it is unclear to what extent anesthesia used in some of these studies may have affected the results, since such procedures are known to dramatically alter blood flow, permeability measures (Goldman and Sapirstein, 1973), and the neuroexcitatory effects of convulsants. Also, it has been unclear whether such circulatory changes can be produced with subconvulsive doses of seizure-inducing agents.

Therefore, cerebrovascular functions were characterized in unanesthetized, unrestrained rats. Fifteen brain regions were examined, including the amygdala, piriform cortex, and hippocampus. These limbic regions were of particular interest because they show the largest decreases in glucose metabolism following limbic seizures (Ben-Ari et al., 1981). It is the piriform cortex-amygdala complex which appears to be critical for the development of limbic seizures (McIntyre and Racine, 1986). This complex is also the site at which neuronal cell loss and glial proliferation occur following prolonged seizures (Engel, 1983), including those produced by

cholinergic agents (i.e., oxotremorine, pilocarpine, carbachol, physostigmine [Olney et al., 1983]). These are some of the same regions affected by soman-induced convulsions (McDonough et al., 1986).

Pathological changes in the central nervous system have been associated with seizures induced with pentylenetetrazol and other agents. These changes include swelling of astrocytic processes, especially around capillaries, which appears to interfere with capillary function (De Robertis, 1960). Subconvulsant, as well as convulsant doses of PTZ are sufficient to alter glial processes (Rodin et al., 1979). Neuronal processes typically appear intact (Rodin et al., 1979). We have completed examination of histologically representative brain samples from rats 3 days following PTZ and soman administration. In the studies described here, we used Fink-Heimer staining procedures to document potential pathological changes following all administrations of subconvulsant, convulsant and treatment drugs.

METHODS

Materials.

The following isotopes and drugs were used in these studies: ^{3}H-sucrose (14 Ci/mmol): NEN Products/Dupont, Boston, MA; ^{14}C-sucrose (350 mCi/mmol): ICN, Irvine, CA; ^{14}C-iodoantipyrine (58 mCi/mmol): NEN Products, Dupont, Boston, MA; pentylenetetrazole (PTZ), Sigma Chemicals, St. Louis, MO; soman, (USAMRICD). Atropine (Butler, Columbus, OH), an oxime cholinesterase activator, HI6 (USAMRICD), diazepam (Hoffmann-La Roche, Nutley, NJ) and a potent relatively non-toxic, neuroprotective agent developed by us (GMM_2) were used in anticonvulsant treatment schedules described below. Tripolar EEG electrodes (MS 303/2) were obtained from Plastic Products One (Roanoke, VA).

Subjects.

Adult male Sprague-Dawley rats (380-430 g) were used for all experiments. Animals were purchased from Harlan-Sprague Dawley Co.(Portage, MI). Animals were group-housed (3/cage) until use. Thereafter, they were housed individually for observation over the experimental period. They were housed in a controlled environment under a 12-hr light:dark light cycle. All experiments have been reviewed by the Wayne State University Animal Care Committee and have met or exceeded AAALAC and DHEW standards as defined in the Guide for Care and Use of Laboratory Animals (DHEW Publication No. (NIH) 78-23).

Dose-response relationships: soman and pentylenetetrazol.

At the beginning of these experiments dose-response relationships (ED_{50}/LD_{50}) were determined for both convulsants. One hundred and twenty male Sprague-Dawley rats (380-430 g) were surgically anesthetized (pentobarbital, 45 mg/kg, ip) and implanted with chronic, epidural, tripolar electrodes for determining seizure thresholds. The animals were given at least 1 week to recover from surgery. They were then injected with various amounts of either pentylenetetrazol (intraperitoneal) or soman (subcutaneous), utilizing stock solutions of 50 mg/ml or 100 μg/ml in saline, respectively. All animals were scored behaviorally and recorded electrographically in these dose-response determinations.

Behavioral scoring of seizure severity was modified from that of Jimmerson (1989). Score 0 = symptom free; score 1 = ataxia, licking & chewing, and/or muscle fasciculation; score 2 = tremors, salivation, limb weakness with hind limb splaying, respiratory distress and/or convulsions; score 3 = moribund with loss of righting reflex.

Treatment schedules.

In the latter half of this project, cerebrovascular studies were conducted in animals injected just prior to a convulsant dose of pentylenetetrazol or soman with either an intraperitoneal injection of a triplet of anticonvulsant drugs ("triple-treat," [TT]) containing atropine (15 mg/kg), the oxime HI6 (1-[[[4-(aminocarbonyl) pyridino]-methoxy] methyl]-2-[(hydroxyimino) methyl] pyridinium dichloride) (125 mg/kg), and diazepam (2 mg/kg), or the triplet supplemented with GMM_2 (70 μg/kg). Five to six hours after these injections animals received an additional 70 μg/kg dose of GMM_2. This latter quadruplet drug combination is referred to as "Q+". These doses of TT and Q+ have been shown by us to reliably prevent convulsions (>75%) produced by a 55-mg/kg dose (1.3 x ED_{50}) of pentylenetetrazol or by a 90 μg/kg (1.3 x ED_{50}, 1.2 x LD_{50}) dose of soman.

Calculations for cerebrovascular measurement.

Procedures were developed which permitted the simultaneous measurements of permeability-capillary surface products and vascular spaces to multiple regions of conscious, unrestrained rats. We combined Rapoport's methods for measurement of brain permeability, employing the 10-min uptake of ^{3}H-sucrose as a diffusion-limited marker (Rapoport et al., 1980), in concert with the 20 sec regional content of ^{14}C-sucrose as a measure of the brain vascular space (BVS). Regional brain blood flow was measured using a new version of our own method (Goldman and Sapirstein,

1973), which employed the fractionation of a bolus of ^{14}C-iodoantipyrine, and included a correction for the time-dependent efflux of the tracer from brain tissues. A "dye"-dilution technique was used for the measurement of cardiac output, in which this same isotope replaces conventional dyes (Sapirstein, 1958).

Blood flows and permeabilities were measured in 15 brain regions, as follows: olfactory bulb, olfactory tubercle, occipital cortex, parietal cortex, frontal cortex, piriform cortex-amygdala, basal ganglia, hippocampus, inferior and superior colliculus, hypothalamus, septal area, midbrain (including thalamus), pons/medulla, and cerebellum. Cardiac output as well as pH, $PaCO_2$ and PaO_2 of the arterial blood and hematocrit were monitored at the same time. Blood pressure was monitored in subsets of 4 animals during a typical moderate seizure (<10 min duration) and for several hours thereafter. Additional subsets of 4 animals were studied after subconvulsant injections. Catheterization and handling of these animals were identical to those in which cerebrovascular measurements were determined.

Blood flow calculations. The method (Goldman and Sapirstein, 1973) assumes that the indicator of flow is completely extracted and that the tissue reservoir into which the indicator flows is sufficiently large compared to the influx rate so that the efflux is not significant during the period of measurement, i.e., about 20 sec after intravenous delivery or about 11 sec after peak arterial concentrations have been reached in the brain. As we have previously described in detail (Goldman and Sapirstein, 1973), under these conditions the rather complex Kety equation and the associated Sokoloff method (Sakurada et al., 1978) can be simplified considerably. The revised equation, which is the basic equation of the indicator-fractional technique (Sapirstein, 1958), is written:

$$U(i)/I = F(i)/CO \text{ when } 6<T<30 \text{ sec} \qquad [1]$$

where
$U(i)$ = tissue uptake of indicator
I = the injected dose of indicator
$F(i)$ = the blood flow in the tissue
CO = the cardiac output

Equation [1] states, in effect, that when a highly diffusible indicator, such as iodoantipyrine, is administered in a single intravenous injection and the killing time is short, then the pattern of indicator distribution in the brain will be the same as the pattern of the fractional distribution of the cardiac output. Since a single intravenous injection is substituted for the complex continuous infusion in the Kety method and since this can be accomplished under essentially non-traumatic circumstances, the single-injection technique makes possible more responsive blood-flow values in conscious, unrestrained animals.

The most recent modification of the indicator fractionation technique recognizes the fact that the rate of efflux of iodoantipyrine is great enough at T=15 sec so as to reduce tissue content significantly and thereby the estimate of blood flow; the error is proportional to the blood flow (Eckman et al., 1975; Raichle et al., 1976). At the killing time of 15 sec after intravenous injection of these tracers we have found that rCBF is underestimated by about 17% in unanesthetized, unrestrained rats (unpublished observations).

Cardiac output measures. Additionally, integration of the arterial concentration of the reference indicator, iodoantipyrine, can be used to calculate the cardiac output (CO) by indicator-dilution according to the equation:

$$CO = J/\int_0^T Ca'\ dt \qquad [2]$$

where J is the amount of indicator injected, and Ca' is the 15-sec integrated arterial concentration of the indicator, corrected for recirculation.

Regional cerebral permeability calculations. Regional permeability of sucrose is estimated by the method of Rapoport, which is published in detail elsewhere (Ohno et al., 1978; Rapoport et al., 1980). Its use in conscious, unrestrained rats is described in the protocol below. The estimate of permeability, the PS (s^{-1}), for sucrose is the product of cerebrovascular permeability P ($cm \cdot s^{-1}$) and the capillary surface area S, estimated to be 240 $cm^2 \cdot g^{-1}$ or cm^{-1} in the rat (Crone, 1963). Radiolabeled sucrose is injected intravenously, and the arterial plasma concentration is monitored until the animal is killed 10 min later, time T, so that only a small amount of the poorly diffusible tracer is accumulated in the brain. Brain concentration remains insignificant relative to plasma concentration, and back diffusion from brain to plasma can be ignored in this time period. Under these circumstances, brain uptake of the tracer can be given, as follows:

$$dC_{brain}/dt = PS \cdot C_{plasma} \qquad [3]$$

Integration of equation [3] to time T gives PS in terms of $C_{brain}(T)$ and the plasma concentration integral

$$PS = C_{brain}(T) / \int_0^T C_{plasma}\ dt \qquad [4]$$

C_{brain} in equation [4] represents parenchymal (extravascular) brain concentration of the tracer at the time of death, T = 10 min, and equals net brain concentration minus intravascular content of the tracer. The latter term is the product of the whole-blood concentration and the regional blood volume. In our protocol, regional-

blood volume, BVS, is defined as the sucrose space at 20 sec after intravenous injection. Under the conditions of these experiments, rPS and BVS were determined concurrently in each animal for each experimental condition utilizing modifications of Rapoport's methods (Ohno et al., 1978; Rapoport, et al. 1980) with two differentially labeled forms of sucrose.

Experimental protocols.

Regional cerebral blood flow. Flow was measured 1 hr, 1 day or 1 week after injection of a convulsant drug in conscious animals which were neither chemically nor physically restrained. Three days prior to cerebrovascular measurements, femoral vein and arterial catheters were surgically implanted under halothane anesthesia so that the experiments could be conducted in animals which were essentially free of the effects of anesthetic. On the day of the experiment, the stored catheters were exposed and arterial blood samples were collected for hematocrit, pH, $PaCO_2$ and PaO_2 assays. A single bolus containing isotonic saline and 4 μCi ^{14}C-iodoantipyrine was injected intravenously. Arterial blood was sampled at 15 μl/sec for 15 sec to establish the arterial concentration curve for the indicator. At 15 sec, animals were killed with an iv injection of a bolus of saturated KCl solution (250 μl). Brains were quickly removed and rapidly dissected on a chilled steel plate; brain regions were weighed electronically and prepared for counting. Tissue and blood contents of tracers were readily extracted in Bray's scintillation cocktail without further treatment (>98%) and were counted in a quench-correcting multi-channel scintillation spectrometer (Packard 4530). Tissue content of ^{14}C-iodoantipyrine relative to the integrated plasma content of the indicator (corrected for efflux) represented the flow-fraction of the cardiac output which perfused the tissue. The simultaneous derivation of the cardiac output (Eq. 2) permitted the calculation of regional blood flow.

Permeability. The protocol for the permeability experiments requires the same surgical preparation employed in the blood flow experiments. At the appropriate time period arterial blood was assayed for hematocrit and blood-gas parameters. A bolus containing 10-20 μCi of ^{3}H-sucrose tracer was injected intravenously. This was followed by collection of 17 precisely timed, arterial samples over a 10-min period to determine the integrated plasma content of tracer. At the end of this 10-min period, regional brain vascular spaces were measured by an additional intravenous injection of 4 μCi of ^{14}C-sucrose. Arterial blood was sampled (about 50 μl) every second for an additional 20 sec, at which time the animal was killed with a rapid intravenous injection of saturated KCl. The regional blood volume was estimated from a knowledge of the 20-sec ^{14}C-sucrose contents in brain regions and arterial blood at the time of death. Thus, both permeability and BVS were determined simultaneously according to our dual isotope modification of Rapoport's

methods (Ohno, 1978, Rapoport, 1980). Brains were removed quickly after death and 15 regions were dissected on a chilled steel plate. Regional tissue uptake of ^{3}H-sucrose (corrected for intravascular content) relative to the 10 min integrated plasma content of sucrose served as a measure of rPS.

Electrophysiological recording.

Rats were anesthetized (45 mg/kg, pentobarbital, ip) and implanted bilaterally with cortical screw-type recording electrodes over the frontal and parietal cortex, as well as with an electrode used as ground. Care was taken to avoid damaging the dura or cortex. Connecting leads were soldered to the screw-electrodes and attached to a connecting socket. The entire assembly was anchored to the skull by acrylic cement. Field-Effect transistors (FETS) were used to minimize movement artifact. Recording leads were connected through a mercury swivel to allow relatively unrestricted movement of the implanted animals during recording. Animals were allowed 1 week to recover from surgery before drug treatment.

Prepared animals were placed in a sound-attenuated, electrically shielded recording chamber. Leads from a Grass Model 7 polygraph (Grass Instruments, Quincy, MA) were attached to the recording electrodes, and 5 min of baseline EEG was recorded. Differential recordings were taken between the frontal and parietal cortex areas. Animals were then injected with drug. One hour of continuous EEG recording was carried out, followed by intermittent recording, until seizure activity was terminated or the death of the animal occurred.

Histopathology.

All histopathological analyses were performed by Dr. Hazlett, who was unaware of the drug treatment or animal conditions. The histopathological technique chosen for this project, the Fink-Heimer technique, is a sensitive, selective silver-impregnation procedure which permits the visualization of degenerating axons of all calibers, both myelinated and unmyelinated. While this procedure has been performed on selected sections from several specimens, we have additionally processed much of our experimental material by the cupric-silver procedure. This latter technique, when used in conjunction with the Fink-Heimer, demonstrated both neuronal and fiber (axonal) degeneration.

Coded brains from representative samples of each experimental group were examined for axonal and neuronal degeneration. Such animals were deeply anesthetized with sodium pentobarbital (50-60 mg/kg, ip) and were sacrificed by transcardiac perfusion with 100 ml of phosphate-buffered saline, followed by 150-250

ml of 10% buffered formalin. Following perfusion, brains were removed and hardened in additional volumes of fixative for a period of 7-20 days. The brains were cryoprotected in increasing concentrations of sucrose in physiologic saline and sectioned in the frontal plane on a freezing microtome at 10-12 microns and were processed for degenerating neurons and axons.

Seventeen brain regions were routinely examined: medullary reticular formation, cerebellar cortex, deep cerebellar nuclei, inferior and superior colliculi, midbrain reticular formation, occipital, parietal and frontal cortices, hippocampus, ventral hippocampus, hypothalamus (paraventricular n.), septal nuclei, caudate-putamen, amygdala, olfactory tubercle and olfactory bulb. Criteria for cellular abnormality included the appearance of small to medium sized vacuoles within cells or grossly swollen cells with large vacuoles and membrane ruptures. The incidence of such regional cellular abnormality was rated on a scale from 1-4; 1 = no obvious cell damage; 2 = limited (5-10%); 3 = mild to moderate (25%); 4 = severe (>50%).

Data analysis.

Dose-response calculations (ED_{50}, confidence limits) were performed with the Litchfield-Wilcoxon method (1949). Regional cerebral permeabilities, blood flows, vascular spaces and cardiovascular parameters were analyzed by means of univariate and multivariate ANOVA's (BMDP Statistical Software, Inc., Los Angeles, CA). Post-hoc multiple range tests (Student-Neuman-Keul's and Duncan's procedures) were employed to make regional comparisons.

RESULTS

PENTYLENETETRAZOL

Dose-response relationships. The intraperitoneal ED_{50} convulsant dose of PTZ in the Sprague-Dawley rats employed in our studies was 42 mg/kg (95% confidence limits, 36.2 - 48.5 mg/kg); the maximum subconvulsive dose was 26 mg/kg. The dose selected for subsequent studies was 50 mg/kg, since it produced convulsions in a majority of animals (70%) and was not lethal in our population. No animal was injected more than once with this convulsant drug. Potentially lethal seizure episodes at high dose levels (>60 mg/kg) were terminated with sodium pentobarbital (120 mg/kg, ip).

There was a stereotypical progression of behaviors during a moderate PTZ convulsive seizure. The convulsion was preceded by a period of depressed locomotion. The mean latency of the overt convulsive response following the intraperitoneal

injection was 67 ± 4 sec. A convulsion usually began with multiple tonic jerks of the forelimbs (forelimb clonus) and head, accompanied by epileptiform EEG spikes. Within 10 sec of the onset of episodes of forelimb clonus the major ictal period began as the animal exhibited loss of balance and opisthotonus typical of generalized seizures. Often, the loss of balance was preceded by rearing and bilateral forelimb clonus. During the ictal period, animals exhibited various combinations of bilateral hindlimb clonus, clonus of all four limbs, or unilateral clonus, usually of the forelimbs. Chewing occurred throughout the ictal period. Convulsions persisted for an average of 247 ± 14 sec.

Animals having more severe seizures demonstrated short running fits with bursts of violent jumping from 1 to 2 feet off the floor of the chamber. The majority of these animals had multiple seizures, interspersed with varying periods of violent tonic jerks of the forelimbs and head, which were also accompanied by high-amplitude EEG spikes. Although the duration of ictal periods were highly variable and intermittent in this group, the approximate mean duration was 60 min. The animals which attempted to explore interictally did so only with great effort, and appeared unable to support themselves. Interictal EEG spindling was seen in the 4-6 Hz range.

In contrast, behavioral convulsions were not evident in any animal which received a PTZ dose of 25 mg/kg. EEG activity was normal most of the time with occasional departures from low amplitude, high frequency tracings in the form of high amplitude spindling (100 μV) spindling activity of 4-6 sec duration, beginning about 3 min after injection. Spindling episodes reached a maximum of about 6/min at 6 min post-injection and declined thereafter. EEG activity was essentially normal within 10-15 min. Animal behavior was similar to that of saline injected controls, punctuated by periods of exploration and resting activities which were not synchronized with the spindling.

Blood pressure. The standard dose of PTZ adopted for these studies produced (usually) a single moderate seizure (50 mg/kg) within 70 sec after intraperitoneal injection. In a group of four animals, MABP was 119 ± 6 mmHg during a baseline period. A blood pressure spike of 165 ± 4 mmHg, lasting less than 9 sec, was observed at the onset of the convulsion. MABP rose steadily to 139 ± 6 mmHg by 2 min, and to 163 ± 9 mmHg by 4 min; MABP decreased to 134 ± 23 mmHg at 12 min after injection, and baseline values were reestablished within 2 hr.

Blood gases and cardiac output. Significant dose-dependent changes were observed only at 1 hr after either a subconvulsant or convulsant dose of PTZ. Animals were seen to hyperventilate, although general body movements were reduced. Following a subconvulsant injection, arterial blood PaO_2 increased significantly by

11%, $PaCO_2$ increased by 6%, and cardiac outputs rose by 24%, compared to saline-injected control animals. By the next day and at 1 week after convulsions, the blood-gas pattern and cardiac output were normal. Blood-gas parameters also changed after a convulsive seizure, except that their magnitudes were greater; PaO2 increased by 25% and PaCo2 increased by 12%. However, cardiac outputs were unaffected at any post-seizure time.

Regional cerebral blood flows.

Subconvulsive dose (Table 1, Figure 1): By 1 hr after administration of a subconvulsive dose of PTZ (25 mg/kg, .60 ED_{50}), overall rCBF was elevated by about 23% above saline-injected control levels. rCBF was significantly elevated in 11 of 15 brain regions, mainly in the limbic system and associated areas and in structures subserving motor functions. The greatest elevations were seen across all cortical regions (22-32%), in the piriform cortex/amygdala (+30%), septal area (+29%) and basal ganglia (+28%). In contrast, perfusion of both colliculi, hypothalamus, midbrain and pons/medulla were not significantly different from control levels. By 24 hr, overall rCBF was barely elevated (+6%) and perfusion was not signficantly different form controls in any brain region. However, 1 week later, rCBF was increased once again in 6 of the 11 regions which were affected at 1 hr post-injection, i.e., mainly in the occipital and frontal cortex (+17%), piriform cortex/amygdala (+17%), and basal ganglia (+17%).

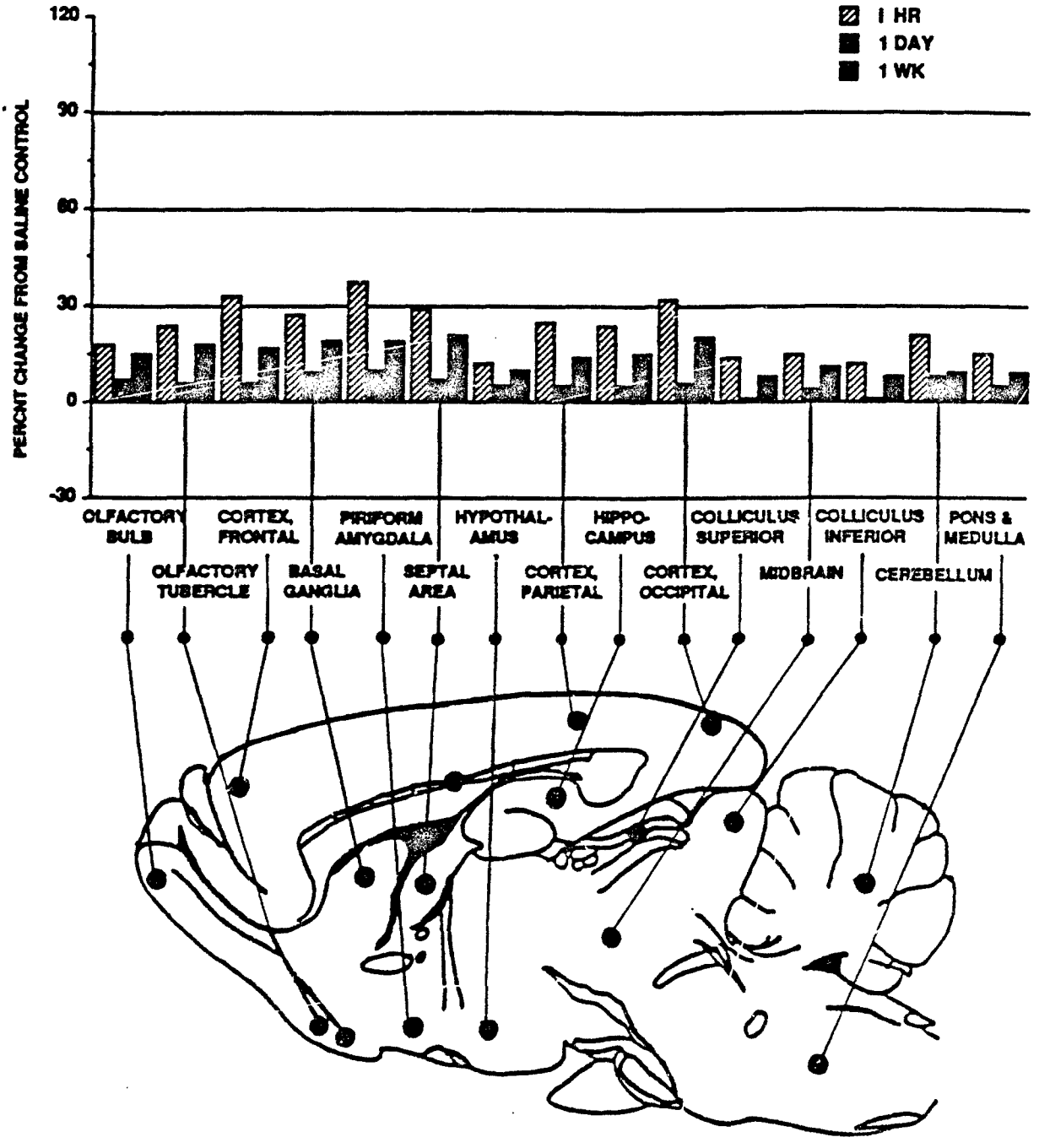
Convulsive dose (Table 2, Figure 2): By 1 hr after injection of a convulsant dose of PTZ (50 mg/kg, 1.20 ED_{50}), overall rCBF was reduced by an average of 5% compared to control levels. In contrast to subconvulsant treatment, a convulsant dose significantly reduced rCBF in the frontal cortex (18%) and in the piriform cortex/amygdala region (27%). rCBF appeared to be unaffected in any brain region at any time period thereafter. These findings are of particular interest because the frontal cortex and piriform cortex-amygdala are both known to be important for seizure processes (McIntyre and Racine, 1968).

Permeability-surface area products (rPS).

Subconvulsive dose (Table 3, Figure 3): By 1 hr after administration of a subconvulsive dose of PTZ, rPS (unlike rCBF) was not significantly affected in any brain region, although average PS rose by 13%. However, by the next day, average rPS was elevated by 37%. PS, unlike rCBF (which had returned to normal levels), increased significantly in the olfactory tubercle (+106%), frontal cortex (+50%), inferior colliculus (+50%), pons/medulla (+50%) and cerebellum (+56%). By 1 week, average PS was **still** elevated by 31%, still significantly in the olfactory tubercle

Figure 1

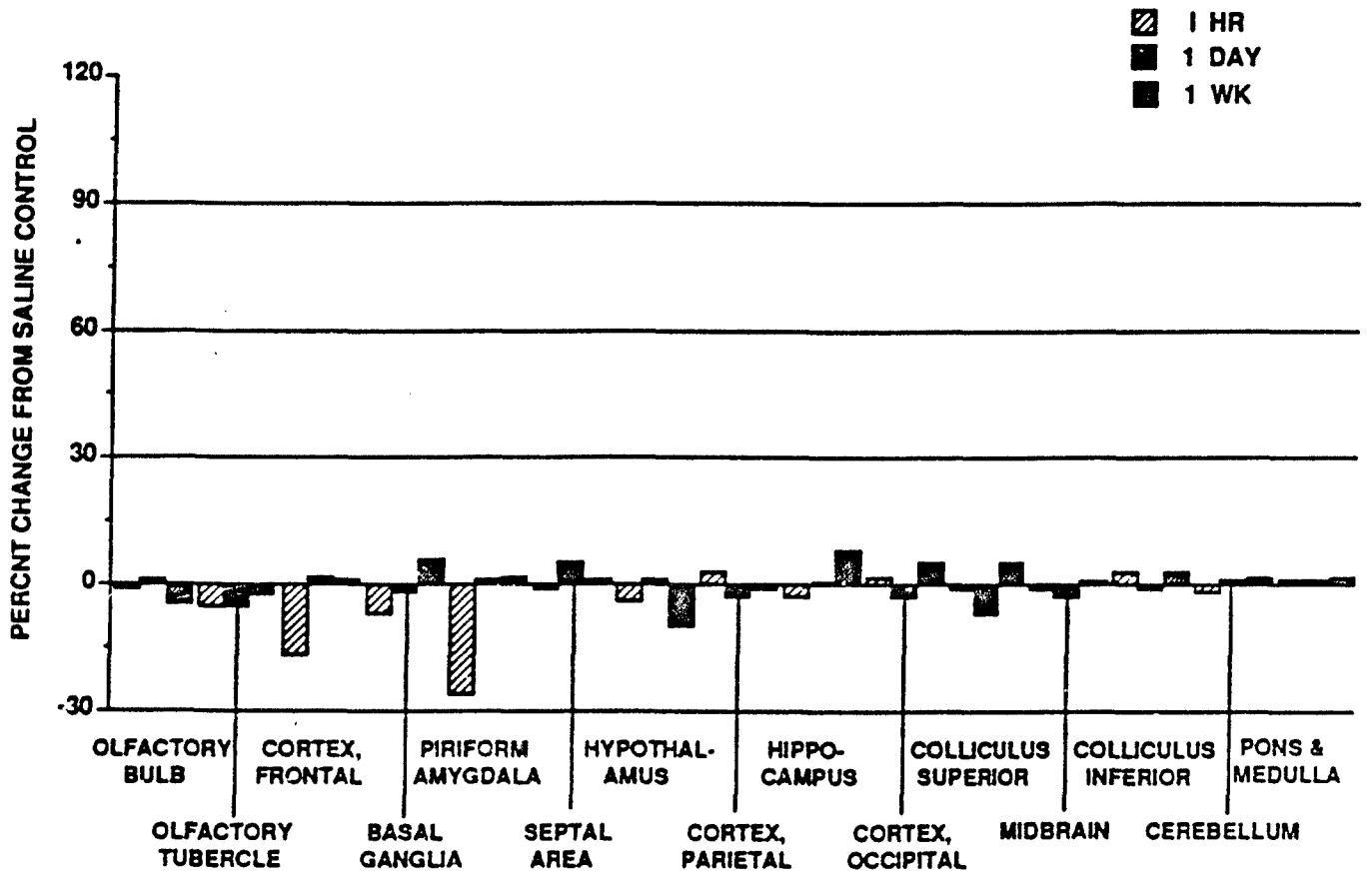
CEREBRAL BLOOD FLOW DIFFERENCES WITH TIME AFTER SUBCONVULSANT TREATMENT WITH PENTYLENETETRAZOL



Consult Tables for levels of statistical significance.

Figure 2

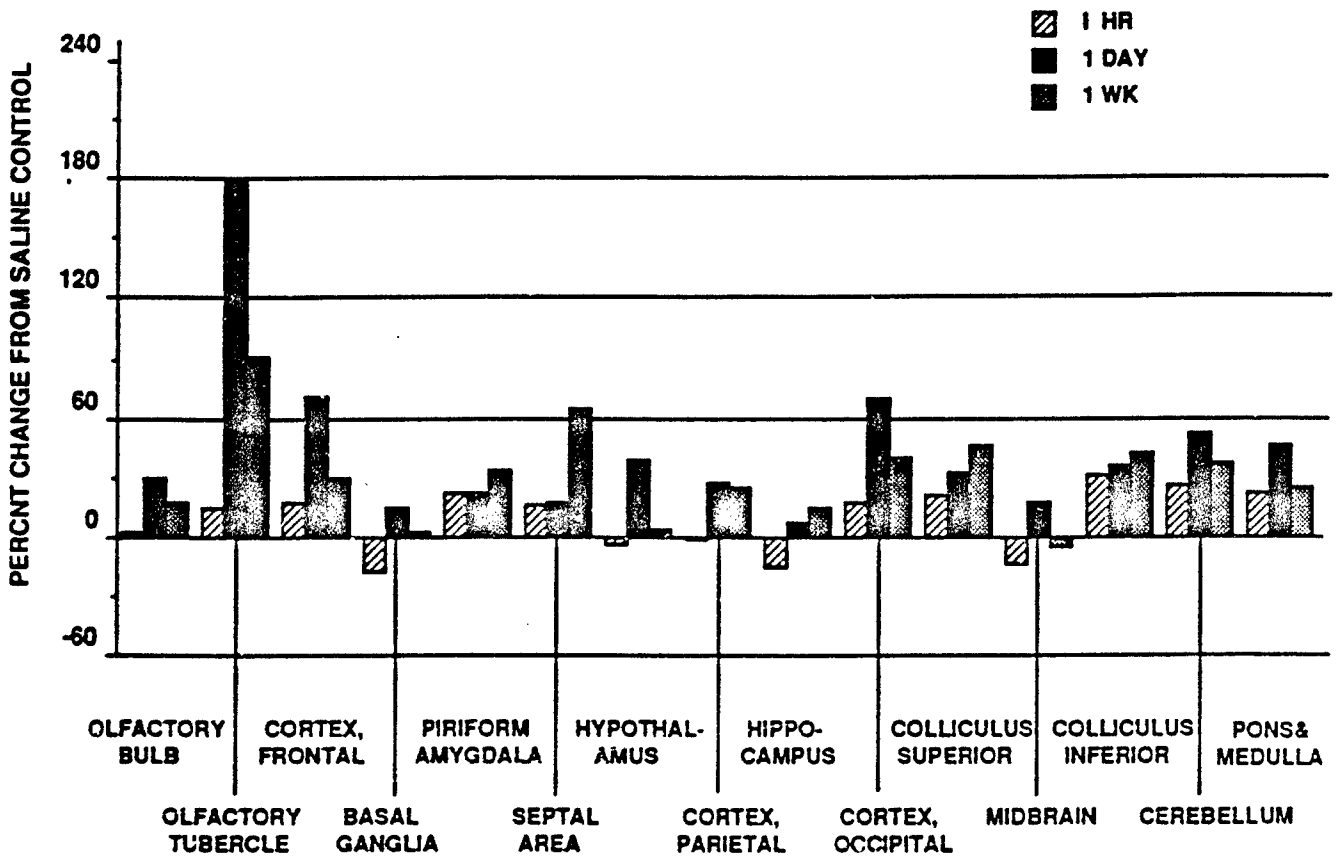
CEREBRAL BLOOD FLOW DIFFERENCES WITH TIME AFTER CONVULSANT TREATMENT WITH PENTYLENETETRAZOL



Consult Tables for levels of statistical significance.

Figure 3

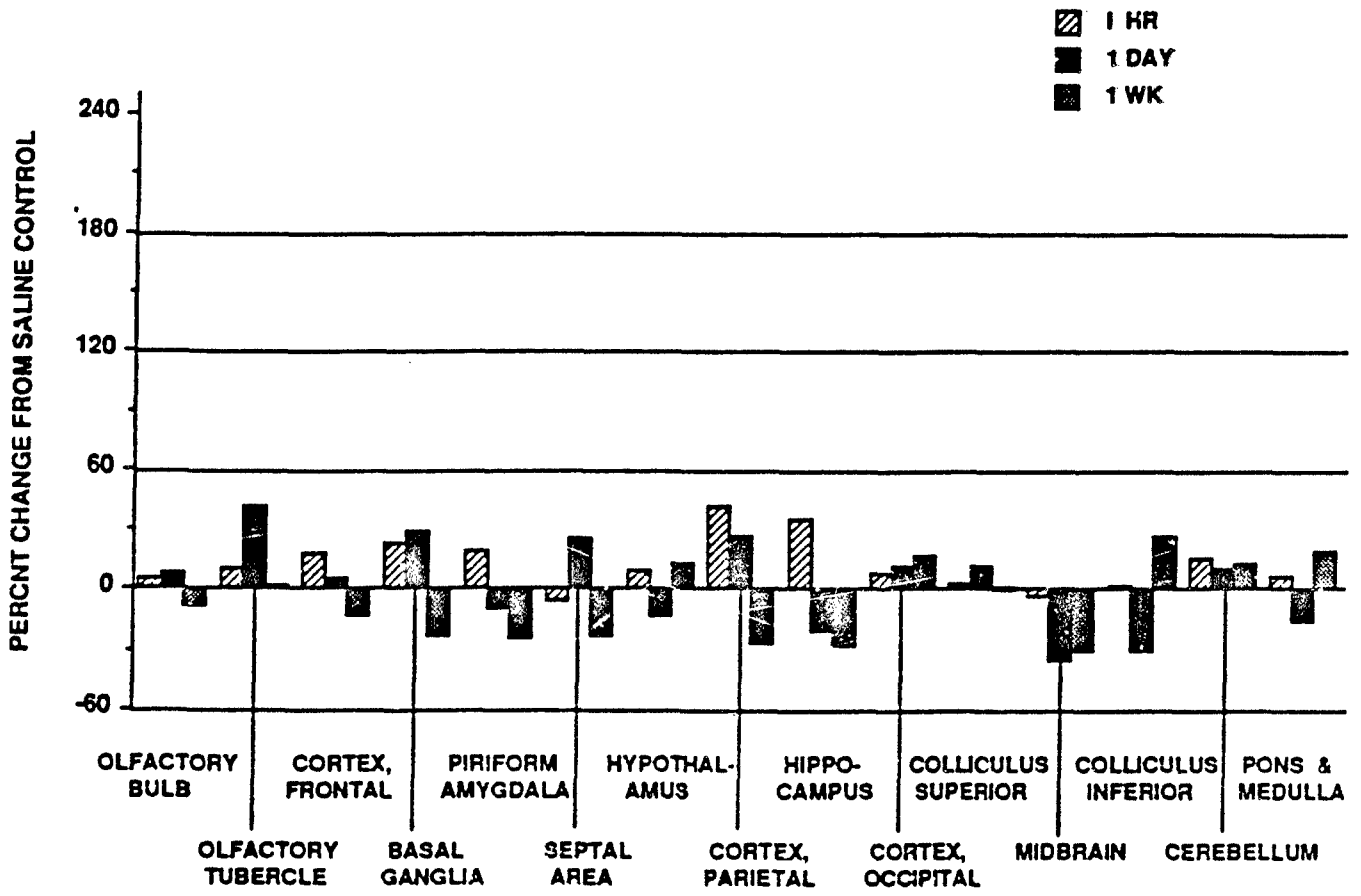
REGIONAL PERMEABILITY DIFFERENCES WITH TIME AFTER SUBCONVULSANT TREATMENT WITH PENTYLENETETRAZOL



Consult Tables for levels of statistical significance.

Figure 4

REGIONAL PERMEABILITY DIFFERENCES WITH TIME AFTER CONVULSANT TREATMENT WITH PENTYLENETETRAZOL



Consult Tables for levels of statistical significance.

(+91%), occipital (+48%) and frontal (+38%) cortices, inferior (+40%) and superior colliculi (+37%) and cerebellum (+38%).

Convulsive dose (Table 4, Figure 4): Although average PS rose 17% during the 1 hr after PTZ administration, rPS levels were not significantly elevated in any brain region. By the next day, average PS remained unaffected (-3%), although some rPS's had begun a downward trend. This was quite unlike results for the same time period after a subconvulsant PTZ dose: in that case rPS was elevated in 6/15 regions. By 1 week, rPS in virtually every brain region was lower than control levels, although not significantly. Again, this was quite unlike the still significant increases in rPS seen after a subconvulsant dose of PTZ.

Regional brain vascular space

Subconvulsive dose (Table 5): Average BVS declined in all three time periods, -12%, -23% and -12%, respectively. Nevertheless, regional differences were significant in only two areas, the parietal cortex (-35%) and in the piriform cortex/amygdala ((-35%), and only on the day after drug administration. Possibly, regional differences were also significant in the septal area.

Convulsive dose (Table 13): Average BVS was essentially unchanged at all time periods. There were no significant regional differences from saline injected control levels.

Histology.

Paralleling rPS effects, the major histopathological effects were observed in animals receiving a *sub*convulsant dose of PTZ. The average injury score for 17 brain regions was 2.3, on a scale of 1-4. Mild cellular damage was observed in 14/17 brain regions: the medullary reticular formation, cerebellar cortex, deep cerebellar nuclei, both colliculi, midbrain reticular formation, occipital, parietal, frontal cortices, hippocampus, septal nuclei, caudate-putamen, amygdala and olfactory tubercle. Little or no damage was seen in the ventral hippocampus, hypothalamus, or olfactory bulb.

Again paralleling rPS effects, little or no brain histopathology was observed in 16/17 brain regions 72 hr after a PTZ-induced convulsion. The sole exception, the septal region, showed severe cellular damage.

SOMAN

Dose-response relationships. The subcutaneous ED_{50} convulsant dose of soman in the Sprague-Dawley rats employed in our studies was 71.5 μ/kg (95% confidence

limits, 64 - 80 μg/kg); the maximum subconvulsive dose was 51 μg/kg. The LD_{50} for 24 hr survival was 75 μg/kg; for 1 week survival, the LD_{50} was about 60 μg/kg. The dose selected for cerebral blood flow and permeability studies was 70 μg/kg, since it produced convulsions in approximately half of the animals and was not lethal (24 hr) in 60-70% of our population. All anticonvulsant treatment experiments were performed in animals which received a soman dose of 90 μg/kg, 1.2 LD_{50}. No animal was injected more than once with soman. In all other experiments, potentially lethal seizure episodes were terminated with sodium pentobarbital (120 mg/kg, ip).

Seizure behavior. No animals injected with 33 μg/kg, sc soman exhibited either behavioral convulsions or significant electrocorticographic seizure activity. The average onset for soman seizures was approximately 10 min for doses of soman between 55 and 81 μg/kg. This latency decreased to 6.5 min at 99 ug/kg of soman. The average seizure onset latency for soman was considerably longer than for PTZ; this was probably the result of the different routes of administration of the two convulsants (i.e., sc versus ip).

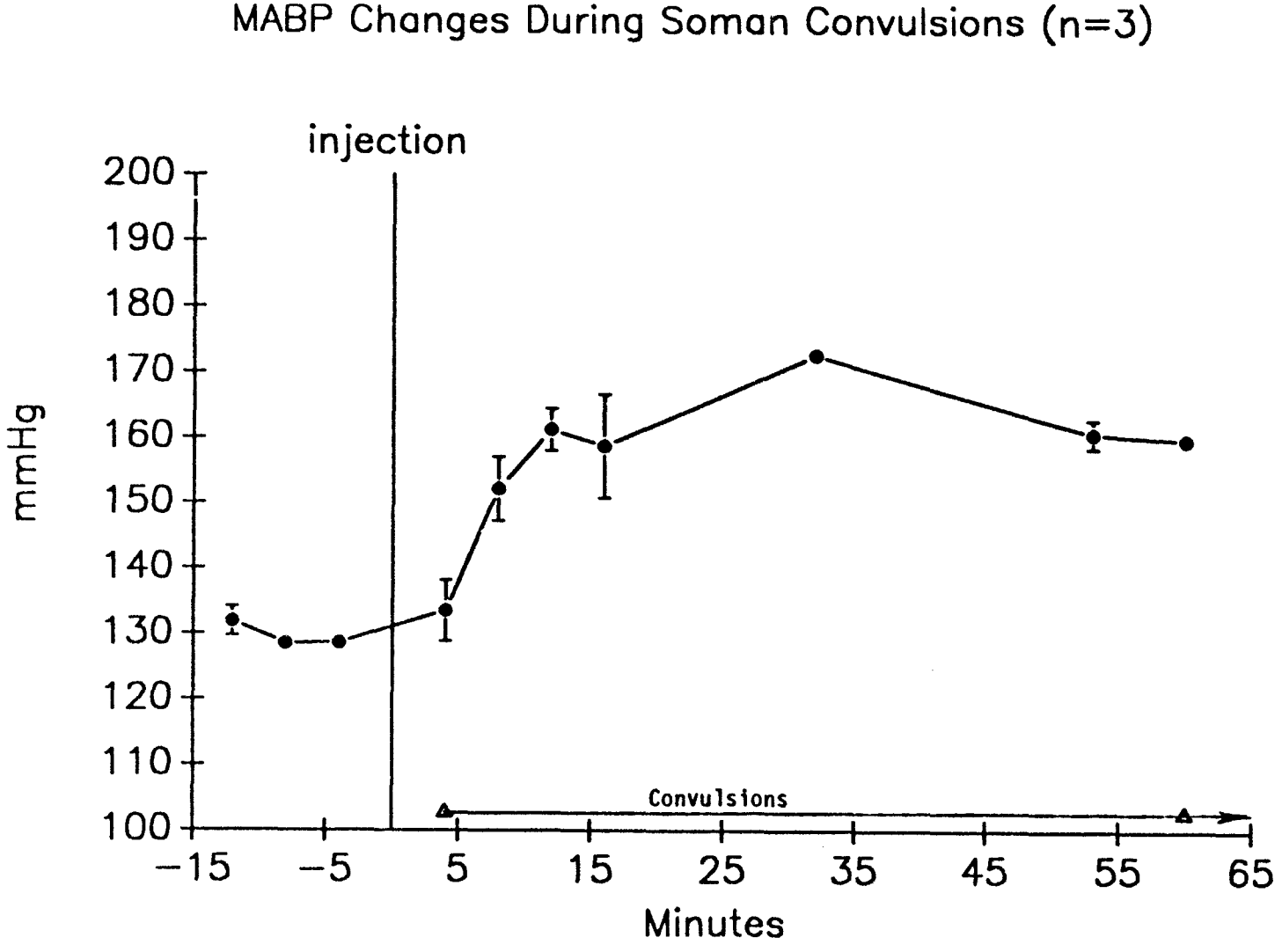
Soman injected animals typically progressed through a series of behavioral changes prior to frank seizure activity, reflecting increasing cholinergic activity. The initial symptoms of licking and chewing occurred within 3-4 min after injection. This was typically followed by muscle fasciculations, tremors, salivation and ataxia. The severity of these symptoms became progressively worse, culminating in convulsive seizures. These seizure typically continued, either status or intermittently over the next 8-12 hrs. Animals that demonstrated status type seizures usually died within 24 hrs without anticonvulsant treatment.

Blood pressure. (Figure 5). In response to a convulsant dose of soman (70 μg/kg), MABP increased within 1 min after injection, and reached a peak of 40-50 mmHG above resting levels within 15-30 min. In contrast to PTZ seizures, MABP remained elevated during the seizure period, which for soman typically lasted 4-6 hr.

Histology. Brains examined 72 hr after administration of a *sub*convulsant dose of soman had an average injury score of 2.44, on a scale from 1-4, as a result of limited to moderate damage found among 17 selected regions (Table 7). However, more severe cellular damage was observed in 6/17 regions: midbrain reticular formation, superior and inferior colliculi, parietal cortex, septal nuclei, and caudate-putamen. Regions least affected (none to limited damage) were the medullary reticular formation, ventral hippocampus, and hypothalamic paraventricular.

Brains similarly examined after a convulsant dose of soman had greater and more widespread cellular damage. The average injury score was about 3 (Table 7). All regions showed some lesions. At least 10/17 regions showed moderate-to-severe

Figure 5


MABP Changes During Soman Convulsions (n=3)
injection
200
190
180
170
160
150
140
130
120
110
100
mmHg
Convulsions
−15
−5
5
15
25
35
45
55
65
Minutes

cellular loss; these were, as before, both colliculi, midbrain reticular formation, parietal cortex, caudate-putamen, and additionally, occipital cortex, frontal cortex, hippocampus, amygdala, olfactory tubercle and olfactory bulb.

Regional cerebral blood flow (rCBF).

Subconvulsive dose (Table 8, Figure 6): By 1 hr after administration of a subconvulsive dose (33 μg/kg, .40 LD_{50}), rCBF was significantly elevated in *every brain region* by an average of 35%. This occurred in the face of a relatively constant cardiac output. Blood flow was highest across all cortical regions (37-53%), as well as in the olfactory tubercle (+37%), basal ganglia (46%), hippocampus (46%) and septal regions (46%). On the following day, no regional blood flow was significantly different from control levels, with average flow differences in the range of 1%. Similarly, at 1 week post injection, there were no significant rCBF differences in any region.

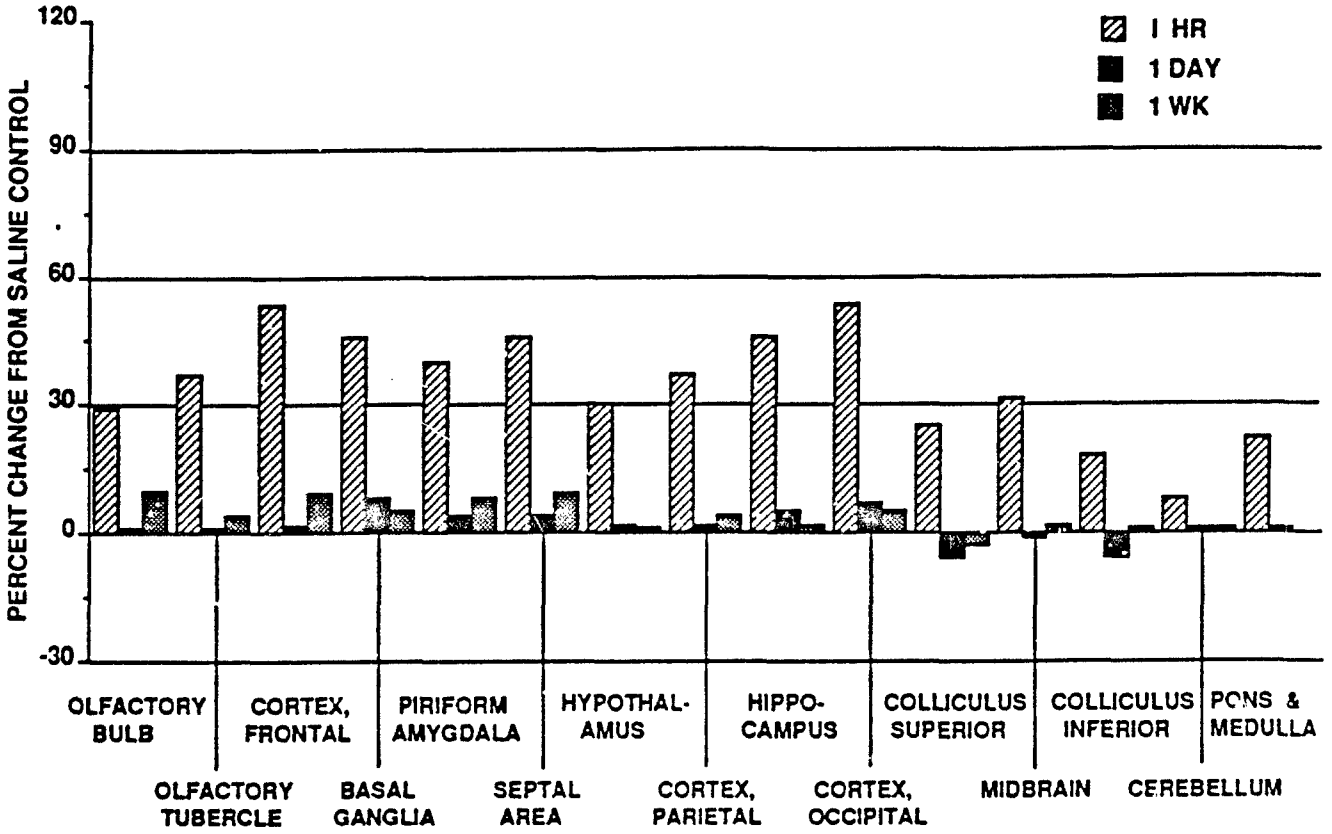
Convulsive dose (Table 9, Figure 7): By 1 hr after administration of a convulsant dose (70 μg/kg, 0.98 x LD_{50}), rCBF was again elevated in *every brain region.* However, the increases were dramatic, averaging 69%. The increase was very great in all cortical areas (52-84%), as well as in the septal area (111%), basal ganglia (95%) hippocampus (89%), piriform cortex/amygdala (84%), hypothalamus (82%) and midbrain/thalamus (74%). There were no significant changes in cardiac outputs at 1 hr. By 24 hr, rCBF was still elevated in 7/15 brain regions; the occipital and parietal cortexes, hippocampus, septal area, hypothalamus, and, especially, the septal area. By contrast, cardiac output was significantly depressed (28%); this effect appeared to be related primarily to reduced physical activity. One week later, rCBF was still elevated in 2/15 regions (septal and basal ganglia), and cardiac output was substantially elevated, paralleling the gross hyperactive motor behavior of these surviving rats.

Permeability-surface area products (rPS).

Subconvulsive dose (Table 10, Figure 8): In contrast to blood flows, by 1 hr after administration of a subconvulsive dose (33 μg/kg, .50 x LD_{50}), the average brain rPS increased by only 10%. However, rPS was significantly elevated in 2/15 brain regions: the pons/medulla and cerebellum, by 57 and 83% above control levels, respectively. On the following day, rPS in the same brain regions were still significantly elevated, by 70 and 74%, respectively. No other regions were affected. However, at 1 week post injection, increased rPS levels were observed in four regions: the olfactory bulb and olfactory tubercle (46 and 73%, respectively), pons/medulla (59%) and cerebellum (61%). By 1 week post-injection, average brain rPS had risen

Figure 6

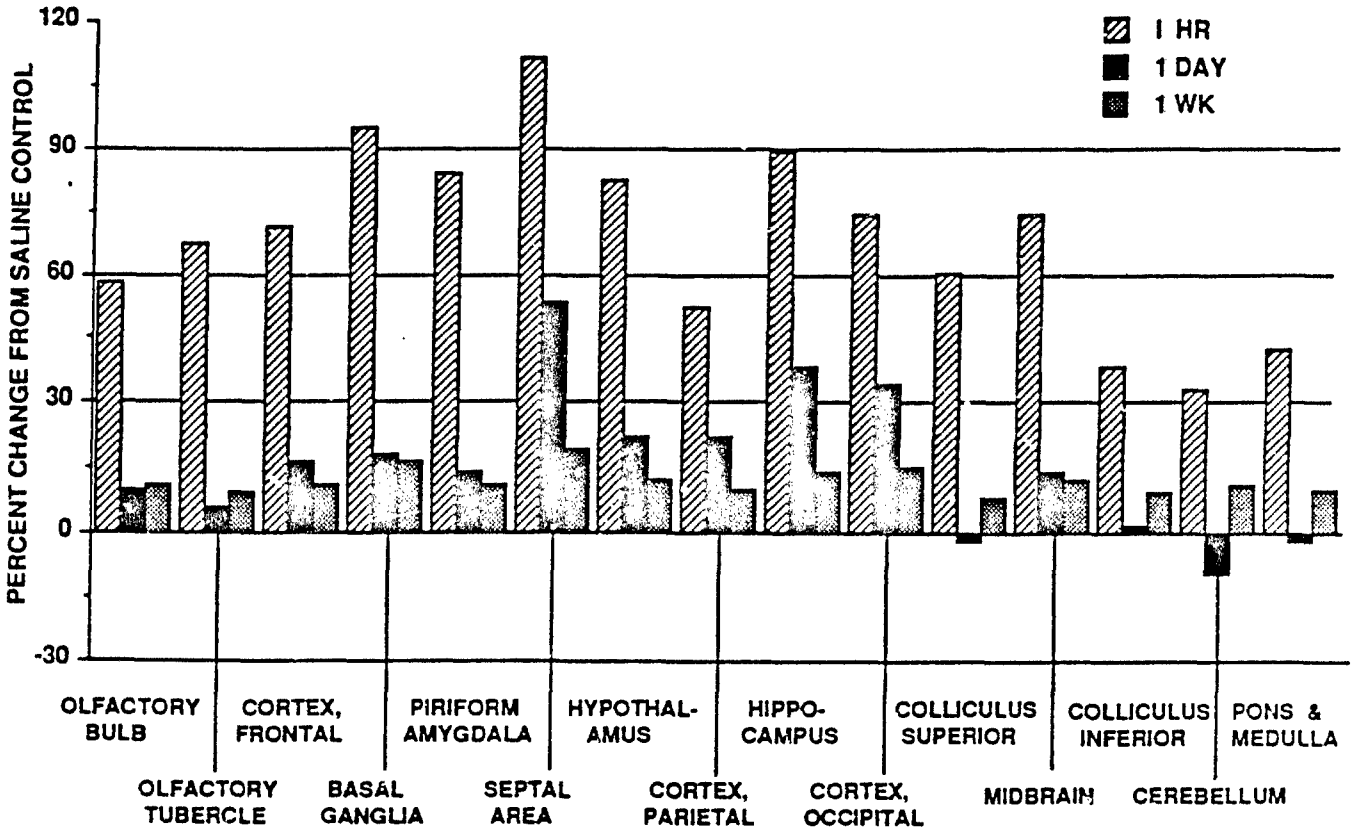
CEREBRAL BLOOD FLOW DIFFERENCES WITH TIME AFTER SUBCONVULSANT TREATMENT WITH SOMAN



Consult Tables for levels of statistical significance.

Figure 7

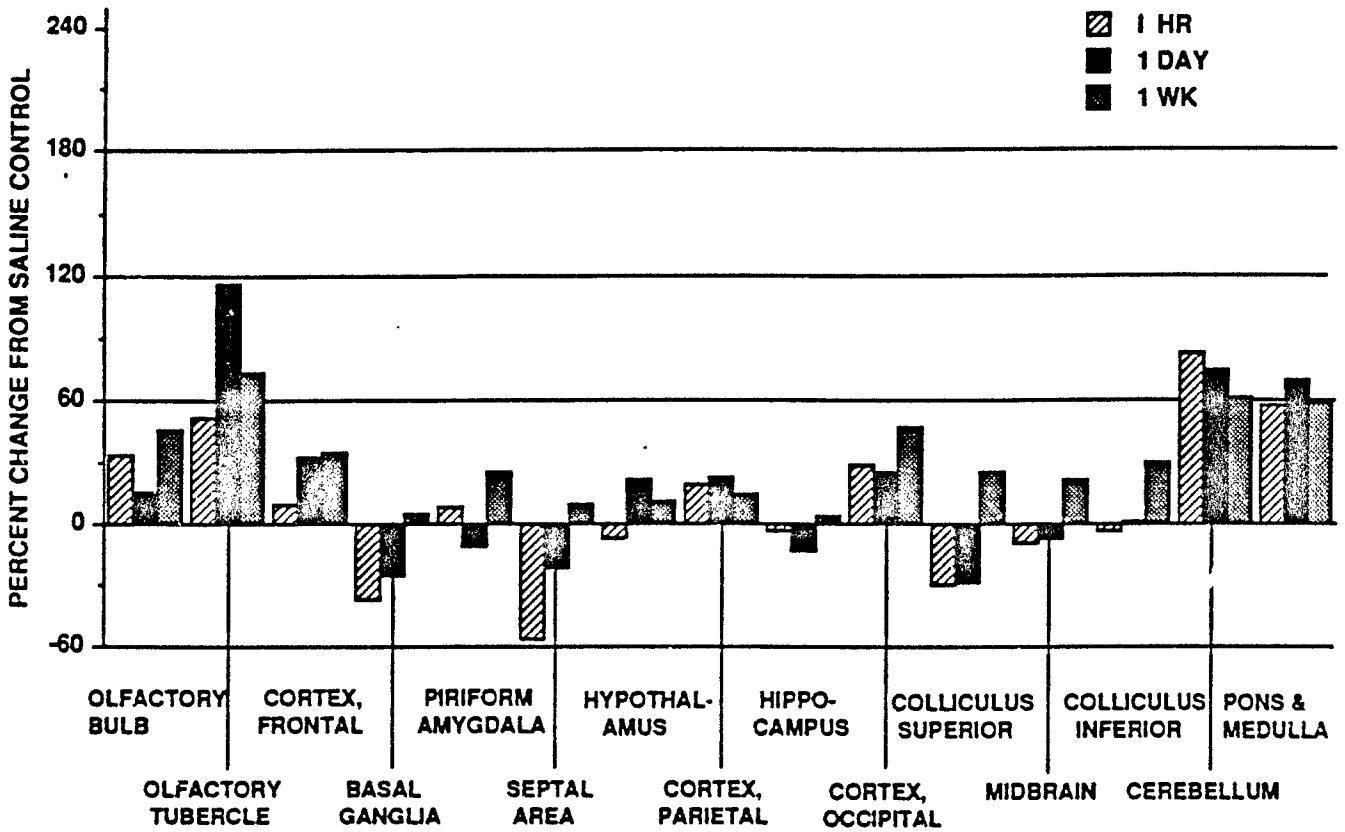
CEREBRAL BLOOD FLOW DIFFERENCES WITH TIME AFTER CONVULSANT TREATMENT WITH SOMAN



Consult Tables for levels of statistical significance.

Figure 8

REGIONAL PERMEABILITY DIFFERENCES WITH TIME AFTER SUBCONVULSANT TREATMENT WITH SOMAN



Consult Tables for levels of statistical significance.

to 31% above control levels.

Convulsive dose (Table 11, Figure 9): In contrast to the generally limited cerebrovascular responses to subconvulsant injections of soman, by 1 hr after administration of a convulsant dose (70 μg/kg, 0.98 x LD_{50}), rPS was massively elevated in *every brain region* by an average of 142% above control levels. Elevations in rPS were highest in the basal ganglia (234%), midbrain (203%), hippocampus and hypothalamus (176%). By the next day, average rPS was elevated by 75%. rPS levels were significantly elevated in 10/15 brain regions: the olfactory tubercle (225%); occipital (241%), parietal (95%), and frontal (218%) cortices; piriform cortex/amygdala (107%); hypothalamus (64%); midbrain (62%); pons/medulla (77%) and cerebellum (65%). At this time, rPS in several brain regions, most notably the superior colliculus (-41%), began to drop below control levels. This trend became clearer by 1 week post-injection as overall rPS dropped to 17% above control levels, with significant elevations still evident in only 4/15 regions. These were the olfactory tubercle (+73%), cerebellum (+61%), pons/medulla (58%) and olfactory bulb (36%).

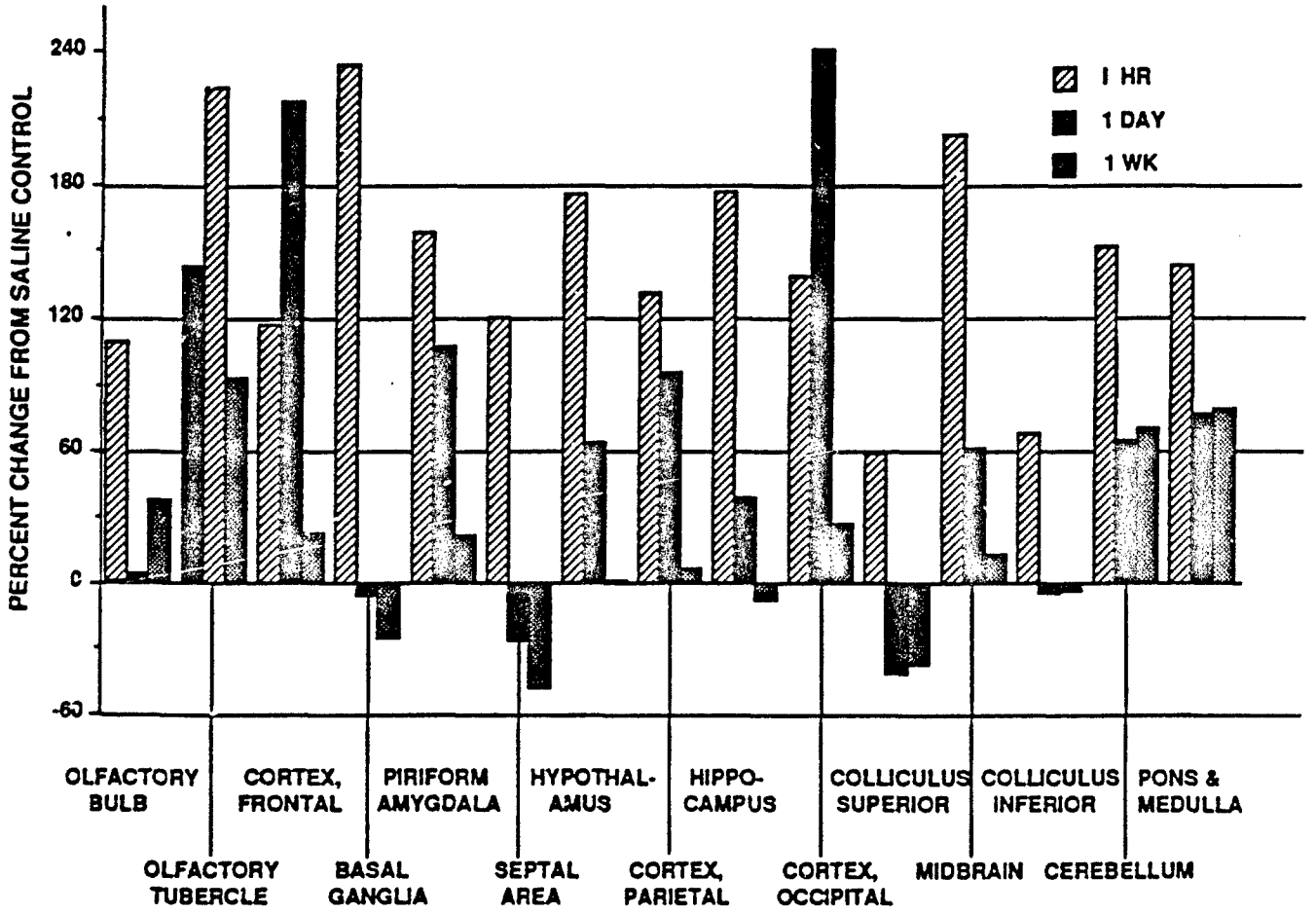
Regional brain vascular space.

Subconvulsive dose (Table 12): Average BVS declined in all time periods: -22% by 1 hr, -24% at 1 day and -8% at 1 week. By 1 hr, BVS was signficantly reduced from control levels in the olfactory bulb (-32%) and cerebellum (-29%). By the next day, these same regions were still affected by the same amount (about -30%). Additionally, BVS declined in the parietal cortex (-37%) and piriform cortex/amygdala (-32%). One week later, intravascular space was still significantly reduced in the piriform cortex/amygdala (-24%).

Convulsive dose (Table 13): A convulsant dose of soman increased overall BVS by an average of +15% by 1 hr after administration; significant increases above control levels were observed in the hippocampus (+36%), superior colliculus (+33%) and septal area (+52%). In contrast, on the day after soman injection, overall BVS levels were reduced by an average of 12%; significantly in the olfactory bulb (-33%) and cerebellum (-36%), while still increased in the occipital cortex (+28%). By 1 week, BVS was reduced in every brain region; average BVS was -11% of saline controls. As observed in animals which received a subconvulsant dose, the largest reductions following a convulsant dose were seen in parietal cortex (-25%) and piriform cortex/amygdala (-13%).

Figure 9

REGIONAL PERMEABILITY DIFFERENCES WITH TIME AFTER CONVULSANT TREATMENT WITH SOMAN



Consult Tables for levels of statistical significance.

Blood parameters.

Subconvulsive dose: Arterial blood pH remained slightly but significantly decreased (-1%) in all time periods, 1 hr - 1 week postinjection. All other circulatory parameters were normal.

Convulsive dose: Again, arterial blood pH remained slightly elevated but significantly reduced in all time periods. However, cardiac output was significantly elevated by 34% above control levels at 1 week postinjection, in parallel with observations of extreme excitability in such animals.

ANTICONVULSANT PRE- AND POST-TREATMENTS

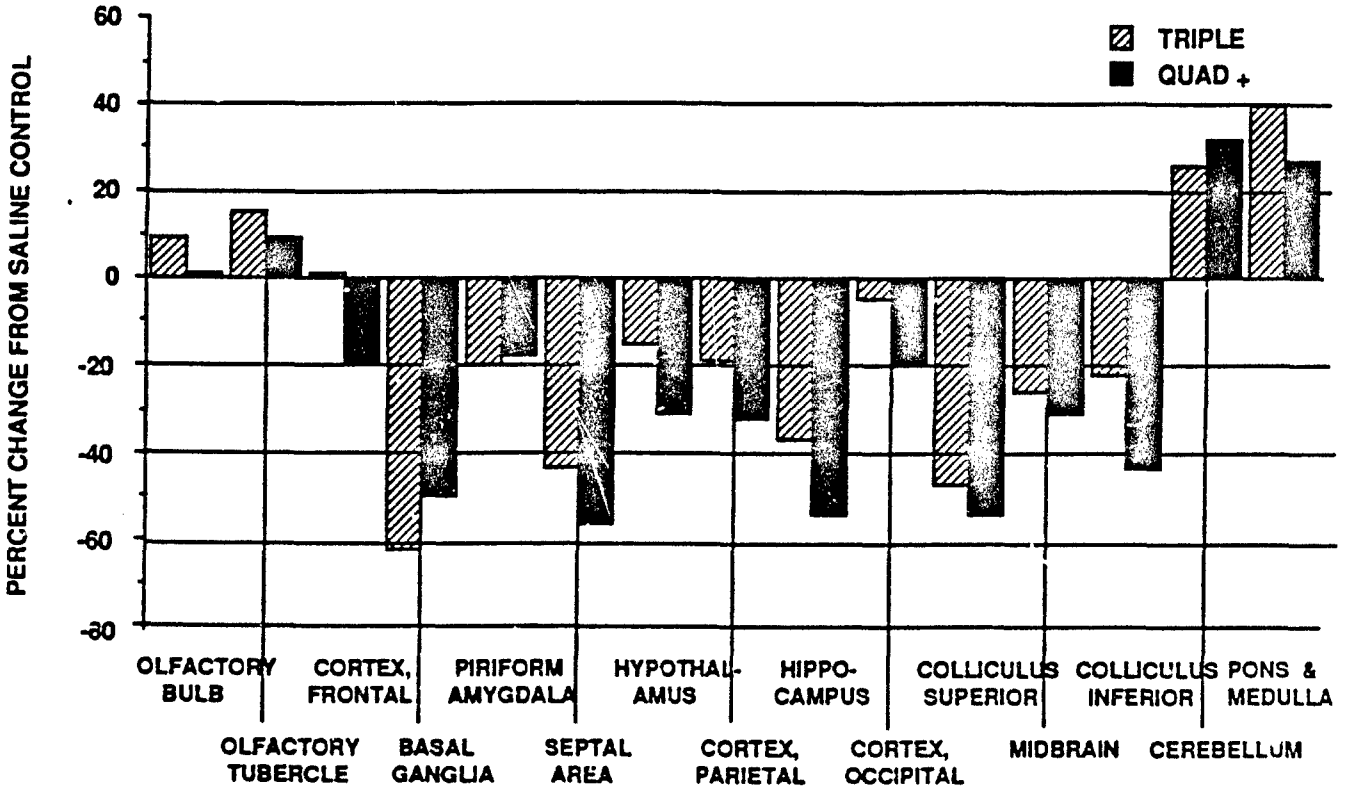
Two types of pretreatments, "triple-treat" (TT) and "quad plus" (Q+) were administered 30 min prior to injections of convulsant doses of PTZ or soman. The composition of Q+ was identical to the TT triplet (atropine, HI6 [Boskovic et al., 1984; Hamilton and Lundy, 1989], and diazepam), with the addition of nanomolar amounts of our proprietary neuroprotective agent, GMM_2. A supplementary injection of GMM_2 was given 5-6 hr after the first, hence the name "quad plus." The dose of GMM_2 was selected on the basis of prior work performed by us on normal rats which showed that an injection of GMM_2, 40 nanomoles/kg intravenously or 50-80 nanomoles/kg subcutaneously, can effectively reduce blood-brain barrier transfer of either sucrose or Rb^{86} by about 30%. The blood t½ of GMM_2 ranges from 20 to 30 min. It has been found that the subcutaneous route provides lower, though considerably more sustained plasma levels. Therefore, the second subcutaneous dose of GMM_2, injected 5 hr after the first, was used to provide a more constant blood level for about 12 hr. All measurements of rPS were made 24 hr after the injection of the convulsant.

rPS effects of TT only, or Q+ only, without convulsant (Table 14, Figure 10). In the TT group, overall PS at 24 hr was lowered by an average of 14%. Significant reductions were observed in 6/15 regions: basal ganglia (-62%), both colliculi (-22 & -47%), septal area (-43%), hippocampus (-37%), and piriform cortex/amygdala (-20%). In the Q+ control group, rPS was reduced in the same regions. There were no significant differences in rPS between the TT and Q+ treatment groups in any brain region.

It should be emphasized here that TT, by itself, not only significantly affected rPS but was fatal in almost 11% of our animals, whereas Q+, by itself, proved fatal only 3% of the time.

Figure 10

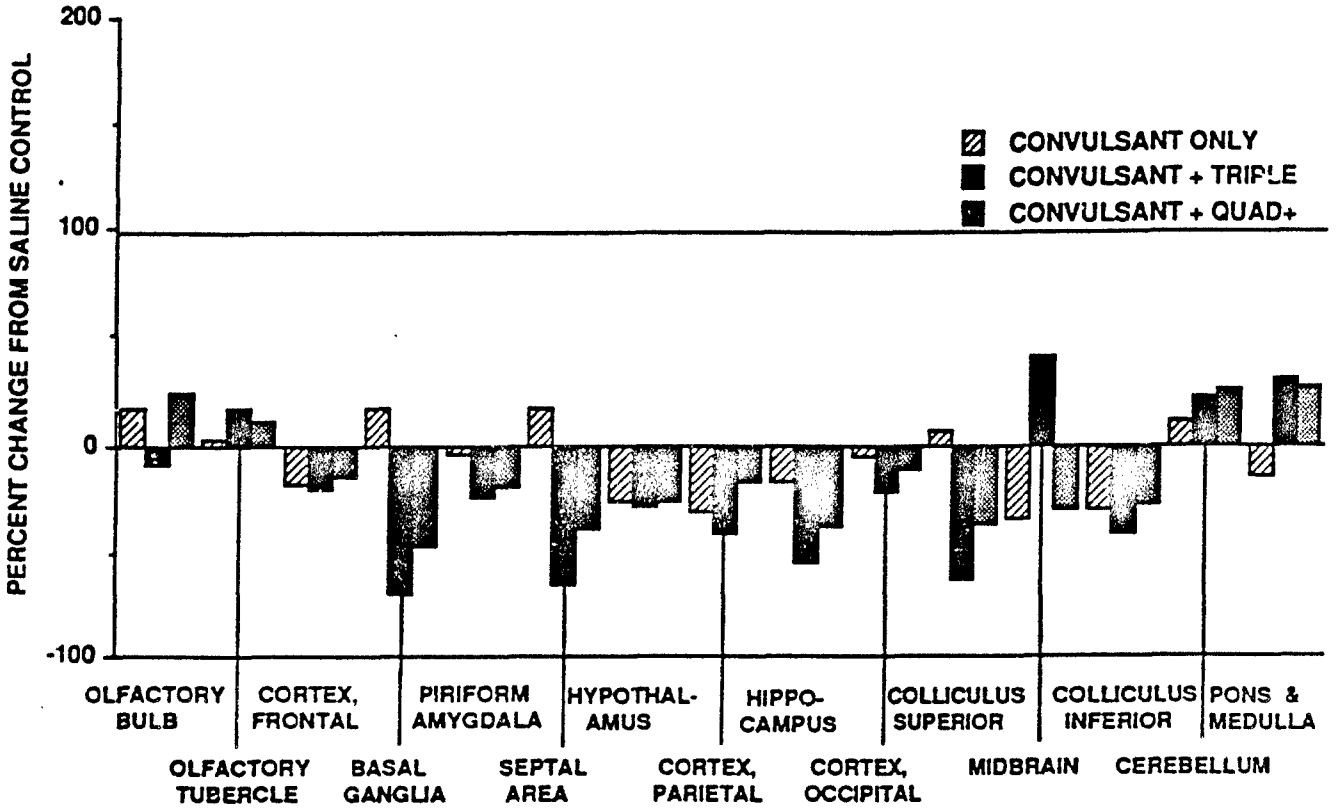
REGIONAL PERMEABILITY MEASURED 24 HR AFTER TREATMENT WITH ANTICONVULSANTS ONLY



Consult Tables for levels of statistical significance.

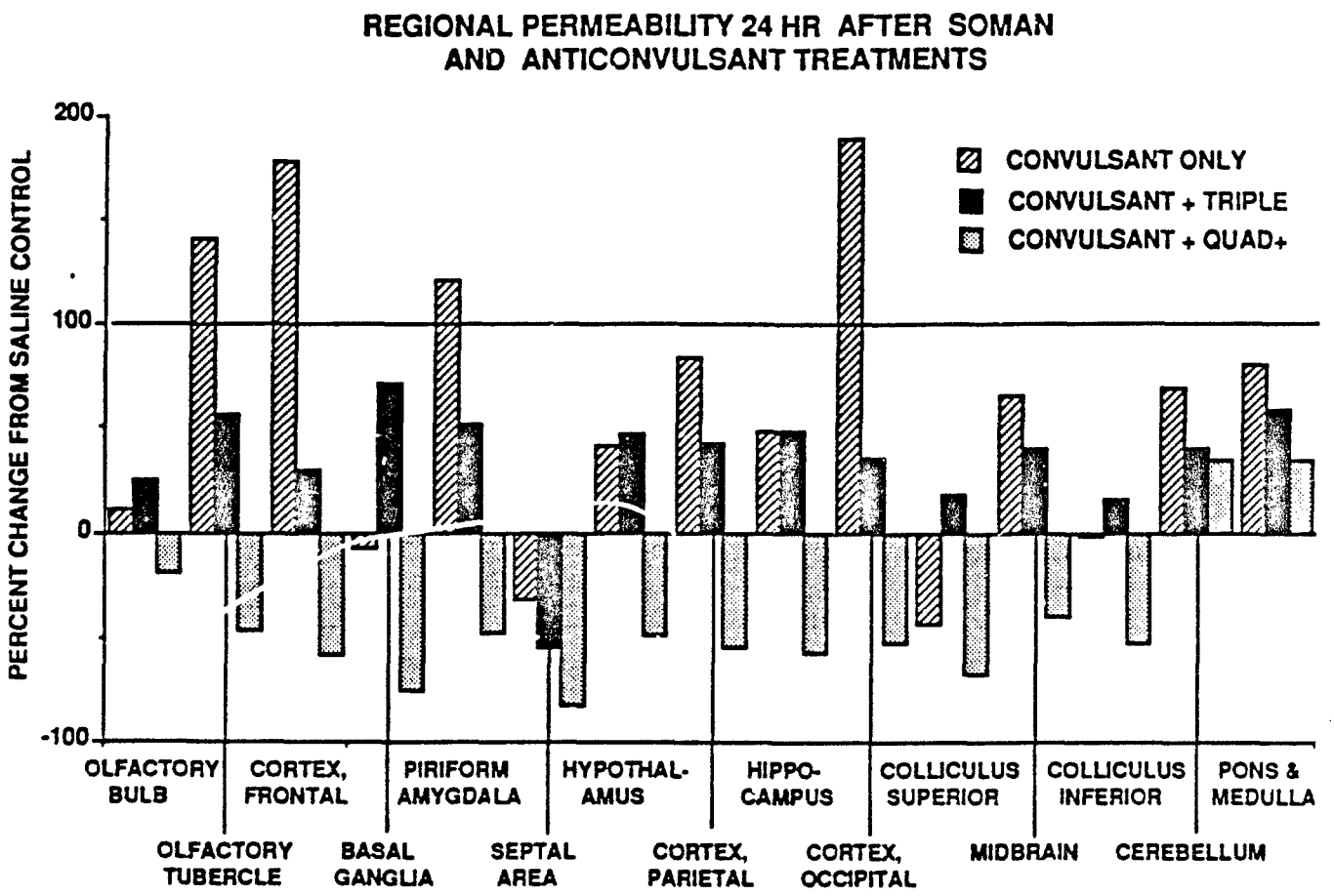
Figure 11

REGIONAL PERMEABILITY DIFFERENCES 24 HR AFTER PENTYLENETETRAZOL AND ANTICONVULSANT TREATMENTS



Consult Tables for levels of statistical significance.

Figure 12



Consult Tables for levels of statistical significance.

rPS effects after treatment of PTZ-induced convulsions. (55 mg/kg = 1.3 ED_{50})(Table 15, Figure 11). Both the TT and Q+ mixtures prevented PTZ-induced convulsions. As noted earlier, in the absence of any protection, there were no significant changes in rPS on the day after a PTZ convulsion. Overall PS was reduced somewhat (by -7%). With the TT anticonvulsant mixture, average rPS was sharply reduced (by -27%). Significant reductions were observed in 6/15 brain regions: parietal cortex (-40%), basal ganglia (-69%), hippocampus (-55%), both colliculi (-40 and -60%), and the septal area (-65%). On the other hand, rPS in the cerebellum was elevated significantly from both the saline control and the convulsed levels. Treatments with Q+ resulted in a similar pattern of reduced rPS, although the magnitude of the reductions were less in every region than with TT pretreatment. However, the differences in rPS between TT and Q+ groups were not statistically significant.

rPS effects after treatment of soman-induced convulsions. (90 μg/kg = 1.2 LD_{50}) (Table 16, Figure 12). Although both TT and Q+ were able to prevent soman convulsions, the protective effects on rPS at 24 hr post-treatment were qualitatively and quantitatively different. It is clear that rPS was severely affected on the day after a convulsant injection; overall PS was elevated 63% from saline control levels and was more than double that in the cortical regions. With TT pretreatment and in the absence of a behavioral convulsion, overall PS was still elevated by an average of +35%, compared to saline control. The differences were significant in occipital (+36%) and parietal (+43%) cortices, piriform cortex/amygdala (+52%), basal ganglia (+72%), hippocampus (+49%), midbrain (+41%), pons/medulla (+59%) and cerebellum (+40%). However, TT appeared to protect the hypothalamus and septal areas, in which rPS was reduced -47% and -55%, respectively from saline control levels.

In contrast to TT pretreatment, the Q+ treatment not only prevented soman convulsions, it also lowered rPS in 13/15 brain regions below saline control levels. PS reductions in most brain regions averaged -38% below saline control levels. Significant "subnormal" rPS levels were observed in occipital (-52%), parietal (-54%) and frontal cortices (-58%), piriform cortex/amygdala (-47%), basal ganglia (-75%), hippocampus (-57%), inferior colliculus (-52%), superior colliculus (-67%), hypothalamus (-49%), septal area (-82%), and midbrain (-39%). Although rPS was still significantly elevated above saline control levels in the pons/medulla and cerebellum (by 35%), these values, nevertheless, were still significantly below untreated convulsed levels.

Thus, it appears that the addition of nanomolar quantities of GMM_2 to the standard treatment triplet of atropine, enzyme activator and benzodiazepine markedly retards the long term rPS changes observed throughout the brain after a substantial convulsant dose (1.2 LD_{50}) of soman.

Histopathology. The cerebrovascular protection afforded by Q+ treatment also was clearly confirmed in the marked reductions in the histopathologic effects of soman toxicity. The average injury score (17 regions) was 1.6, indicating little or no cellular damage. In contrast to TT treatment, after Q+ treatment, only limited damage (5-10%) was observed and only in 5/17 brain regions, including superior colliculus, occipital cortex, septal nuclei, caudate-putamen and amygdala.

Purine Pilot Study:

Preliminary Findings with NECA and Soman:

The objective of this pilot study was to examine the anticonvulsive and neuroprotectant properties of adenosine analogs against soman induced toxicity. *We have found that a stable adenosine analog, 5'N-ethylcarboxamido-adenosine (NECA), reliably blocks soman-induced seizures and death in adult rats at doses of soman which would otherwise be acutely lethal (1.47 and 1.73 LD_{50}). Furthermore, NECA injections prevented or markedly reduced signs of cholinergic activation following soman injections compared to controls. These effects were observed with less than equimolar concentrations of NECA.*

NECA (100 μg/kg which equals 0.32 μmole/kg) injected IP 20 min prior to soman injection (1.47 LD_{50} = 110 μg/kg = 0.6 μmole/kg, sc) prevented both seizures and death in 5/5 mature adult Sprague Dawley rats. **This occurred without benefit of any other anticonvulsant treatments.** Only minor cholinergic symptoms (e.g., salivation) were eventually detected in 2/5 animals, and then only several hours later.

In 2/2 animals, this same dose failed to protect against seizures or death caused by a 1.73 LD_{50} (130 μg/kg = 0.7 μmole/kg) dose of soman. However, seizure onset was delayed by approximately 20 min as compared to that of animals receiving only soman.

With concurrent, equimolar (0.7 μmole/kg) injections of NECA, 1.73-LD_{50} doses of soman failed to elicit either cholinergic symptoms, seizures or death in 2/2 animals. These animals remained healthy and asymptomatic for more than 2 weeks, after which time they were sacrificed for histological examination of their brains.

In this latter group, i.e., NECA-protected against a high soman dose (1.7 LD_{50}), the average histopathological injury score for 17 brain regions was 1.9, indicating little or no overall cell damage (Table 7). However, mild to moderate damage was observed in the inferior colliculus, midbrain reticular formation and caudate-putamen, and severe damage was found in deep cerebellar nuclei.

DISCUSSION

The present studies have yielded several significant observations; most have clear military, as well as basic, implications. A summary of primary objectives of the research and results is listed below and is followed by a general discussion.

1. *Objective*: Examine cerebrovascular effects of soman and PTZ treatments.

Results: We have found marked, time-dependent and regionally specific cerebrovascular effects in experimental animals following soman and PTZ intoxication. Massive increases in both cerebral blood flow and regional blood brain barrier permeability were observed following convulsive doses of soman, with similar, but smaller, effects observed following a subconvulsant dose. Less severe, but more complex patterns of flow and permeability changes were observed following treatment with PTZ. These cerebrovascular disturbances often lasted at least 1 week.

2. *Objective*: Compare the cerebrovascular and neuropathological effects of soman and PTZ.

Results: The regional and temporal patterns of rCBF and, especially, rPS were qualitatively different for soman and PTZ. The effects of soman were present at both subconvulsive and convulsive doses and were much more severe and persistent than those seen after PTZ treatment. In addition, PTZ exhibited an unusual dose-response profile, with greater effects seen after a subconvulsant, rather than convulsant dose.

3. *Objective*: Determine the relationship between cerebrovascular changes following soman treatment and neuropathology.

Results: Cerebrovascular disruption following soman treatment paralleled significant neuronal loss and other cellular abnormalities across a variety of brain regions. Higher doses and larger concomitant disruption of cerebrovascular function were associated with the greatest levels of histopathology.

4. *Objective*: Determine whether or not electrocorticographic seizures *per se* are required in order for neuropathological changes to be produced by soman.

Results: Evidence was found for protracted toxic effects of soman on cerebrovascular function and for marked neuropathology even *in the absence of convulsive behavior or significant cortical electrical seizure activity*. The degree of pathology, however, was clearly greater when convulsive doses of soman were used. Therefore, cortical electrical seizure activity *per se* is not required for soman-induced cerebrovascular pathology. However, occult subcortical seizures cannot be ruled out.

5. *Objective*: Evaluate potential protective effects of a mixture of atropine, HI6 and diazepam (TT) on soman neurotoxicity (e.g., cerebrovascular function and neuropathology).

Results: Pretreatment with TT was effective in blocking behavioral convulsions and electrographic seizures produced with either PTZ or soman. Pretreatment with TT for soman toxicity was only mildly effective in preventing the long-term changes in rPS and histopathology. We also found that treatment with TT alone (i.e., without soman) produced moderate, but significant, cerebrovascular and neuropathological consequences, along with approximately a 11% lethality. A similar mixture, which included the addition of our proprietary neuroprotective agent, GMM2, had no significant neuropathological sequelae and was much less lethal (i.e., < 3%).

6. An additional major finding which has significant basic, medical and military relevance was that the addition of GMM2 to the standard TT treatment (Q+) *was successful in sharply preventing or reversing the soman-induced PS changes in almost every brain region, and almost completely prevented the subsequent histopathology.*

GENERAL DISCUSSION

Convulsant PTZ effects. The results of the present series of experiments have demonstrated that PTZ administration can alter cerebrovascular function. The seizures produced by the dose of PTZ used in these experiments (i.e., 50 mg/kg, ip) were moderate in severity, typically with only a single primary convulsive seizure episode lasting between 2 and 4 min. Seizure onset was marked by sharp, transient blood pressure spike which was followed by a more sustained mean arterial blood pressure (MABP) increase. The magnitude of the increase was substantial, rising from a baseline level of 119 mm to more than 160 mm. This represents a mean increase of almost 40% by the end of the seizure episode, subsiding thereafter. If sustained for more than 10 min, increases in MABP of this magnitude are usually sufficient to open the blood-brain barrier (Ingvar et al., 1984; Johansson & Linder, 1978). With the moderate PTZ seizures in the present studies, the increases in MABP were transient, returning to baseline values within 2 hr. More severe PTZ seizures were accompanied by sustained MABP elevations lasting more than 1 hr. Such seizures, however, were often lethal.

In most brain regions, cerebral blood flows were unchanged at 1 hr after a PTZ seizure. Notable exceptions include the frontal cortex and piriform cortex-amygdala. The significant decreases in these two regions were 18% for the frontal cortex and 27% for the piriform cortex-amygdala. This observation is important because it demonstrates diminished flow, and possibly diminished function, in two brain regions which are critical for seizure induction and which often demonstrate seizure-induced pathology. Support for diminished function comes from studies indicating a decrease in regional glucose metabolism in the mesial-temporal lobe following seizures (Ben-Ari

et al., 1981). The piriform cortex-amygdala also appears to be a generator of epileptiform activity in a variety of seizure models (McIntyre and Racine, 1986). In addition, the piriform cortex-amygdala is the site of major pathology following prolonged seizures, including those produced by soman (McDonough et al., 1986; Engel, 1983). In the present experiments, it is noteworthy that the decreased regional blood flows at 1 hr occurred in the face of reduced arterial pH and elevated pCO_2 levels -- both stimulators of cerebral blood flow -- which may have countered even larger declines in regional flows and functions. Furthermore, the effects of moderate PTZ seizures on blood flow observed in the present studies were transient; i.e., by 15-18 hr and thereafter, regional blood flow had returned to control levels. The possibility exists, therefore, that decreased flow to these critical brain regions may contribute to or mediate the known pathology, particularly following prolonged and severe epileptiform activity.

Under the conditions of the present experiments, regional cerebral permeability was only slightly increased at 1 hr after a PTZ seizure, and the magnitude of the increase did not reach statistical significance for any individual brain region. Permeability did, however, increase in 14 of 16 brain regions by an average of 14%. It is likely that more severe, prolonged seizures would have led to greater changes in permeability, as demonstrated in a previous study following very severe seizures (Ingvar et al., 1984).

As mentioned above, PTZ seizures reduced arterial pH (-11%), elevated $PaCO_2$ (+15%), and substantially increased the PaO_2 (+27%) at 1 hr after a seizure termination. These changes in blood parameters appeared to occur in response to the period of intense skeletal activity and reduced respiration rate during the ictal phase. These parameters returned to baseline levels by 15 hr after seizure termination. At no time interval measured were cardiac outputs significantly altered from control values. Therefore, the peripheral hemodynamics were apparently not responsible for the regional cerebral blood flow changes described above. Similarly, no time-dependent changes in arterial blood hematocrits or body weights were observed.

Subconvulsant PTZ effects. The blood flow responses to a subconvulsant dose of PTZ proved to be more dramatic, not only acutely but also at 1 week post-injection. Flows to 10/15 brain regions increased significantly (by 1 hr), and were essentially normal by the next day, but increased again in 6 of these same regions one week later (Table 1, Figure 1). The most affected regions at both times (1 day and 1 week) were frontal and occipital cortex, piriform cortex/amygdala and the septal area. In contrast, rPS was unchanged acutely but increased in most regions, significantly in 6/15 regions on the day after injection, and was still elevated in 8/15 regions at 1 week. This was accompanied by mild histopathology in 7/17 regions.

In animals which received a convulsant dose of PTZ and in which flow and rPS changes were not significant, histopathology also was minimal.

It is noteworthy that increases in cerebral blood flow and permeability were greater following a subconvulsant dose of PTZ than were those observed after a convulsant dose. Similarly, greater neuropathological changes occurred following subconvulsant PTZ treatment. The explanation for this unusual dose-response relationship for PTZ seizures is unclear. It is possible that higher convulsant, but not subconvulsant, doses of PTZ may mobilize an inhibitory neural system which can counter excitatory effects of PTZ seizures. For example, adenosine release can follow seizure activity and adenosine is known to exert significant cerebrovascular effects and neuroprotection against ischemic brain damage (Phillis and Wu, 1983; Williams, 1984; Dunwiddie, 1985). We are currently investigating this possibility, and recognize that other inhibitory systems also may play a role.

Soman treatments. Soman injections clearly produced major, dose-related changes in cerebrovascular function, as well as dramatic and prolonged electrographic seizures and convulsions. Seizures produced by soman (70 μg/kg, sc) were very severe, with average seizure onset of approximately 8-10 min following subcutaneous injection. Signs of cholinergic activation, including salivation, licking, and muscle fasciculations around the injection site, appeared within minutes of injection and became increasingly more pronounced following soman treatment. These effects invariably preceded seizure activity. Seizures typically began abruptly, with forelimb clonus and EEG spiking; this was followed rapidly by tonic-clonic convulsions and high-amplitude, continuous epileptiform spiking in the EEG. Such seizures continued for long periods of time (4-6 hr) and often became status in nature. During the latter periods of seizure activity, animals were unresponsive, their hindlimbs were splayed, and they exhibited nearly continuous, rhythmic twitching of the muscles of the forelimbs head and neck. The EEG demonstrated intermittent, lower-amplitude, high-frequency spiking during this later period of time. Animals showing these types of seizures typically died within 24-30 hr after seizure onset.

As with PTZ seizures, seizures following soman treatment were marked by a sharp rise in mean arterial blood pressure (almost 50%). However, unlike PTZ seizures, the peak increases in arterial pressure following soman were persistent, lasting more than 1 hr after seizure onset. Clearly, such increases would be sufficient to damage blood-brain barrier integrity (Hardebo and Owman, 1984; Ingvar et al., 1984; Johansson and Lindner, 1978).

Dramatic increases in regional cerebral blood flow were measured following both subconvulsive and convulsive treatment with soman. With subconvulsant levels of soman, large increases in rCBF were observed in virtually all brain regions. Increases ranged from 10 to 55% across brain regions. By the next day, regional blood flows had returned to near normal values, and remained normal at 1 week. In contrast, convulsant levels of soman resulted in dramatic increases in flow in every brain region examined. Such increases ranged from 30% to more than 100% above control levels. In general, rCBF remained elevated across brain regions at 1 day, approaching more normal levels by 1 week. The largest increases in flow following soman treatment occurred in regions with the highest densities of muscarinic cholinergic receptors (Churchill et al., 1985), including the basal ganglia, piriform cortex, septal area, hypothalamus, and hippocampus. The increases in flow following soman were much greater than those produced by PTZ, particularly at 1 hr after seizure. In addition, soman treatment resulted in persistent increases in flow for more than 24 hr, in contrast to the more transient changes seen following PTZ. These persisting flow changes were observed in the septal area, hippocampus and occipital cortex.

One of the most striking effects of convulsant doses of soman was the very large and persistent increases in regional permeability-capillary surface area products. At 1 hr after soman, increases in rPS ranged from 60% to more than 200%. For olfactory tubercle, piriform-amygdala, and all cortical areas (i.e., frontal, parietal and occipital cortex), rPS remained markedly elevated when measured 24 hr later. Even when measured 1 week later, rPS was significantly elevated in hindbrain regions (e.g., cerebellum, pons-medulla). This is clearly unlike the transient rPS changes observed with PTZ.

Histological analyses of brains taken from soman-treated animals clearly demonstrated a high degree of neuronal insult, including substantial cell loss across all 17 brain regions analyzed. These effects were dose-related, with the convulsant dose producing the greatest degree of damage. The greatest evidence of neuropathology following soman was seen in both colliculi, hippocampus, basal ganglia, amygdala and olfactory tubercle. Again, these are areas enriched in muscarinic cholinergic receptors (Churchill et al., 1985). The degree of pathology produced by the subconvulsant dose of soman was uniformly greater than that produced by PTZ at any dose. In general, the pattern of neuropathology following PTZ was relatively uniform across all brain regions, while soman-induced pathology was clearly focused in areas of high cholinergic innervation.

Scremin et al. (1991) have recently reported that subconvulsive doses of soman significantly elevate regional cerebral blood flow measured only at 45 min post-injection time. Curiously, flow was not coupled to indices of glucose metabolism at this time. In these studies, the subconvulsive dose employed, 55 μg/kg, was described as being "non-toxic and non-symptomatic," although no neuropathological evidence was provided. This is in contrast to the present data, which clearly show significant and substantial cerebrovascular and pathological consequences for at least 1 week after administration of subconvulsant doses of soman (33 μg/kg; Table 10, Figure 8). Our findings of significant and long lasting pathophysiology induced with subconvulsant doses of soman support the behavioral studies of Hymowitz et al. (1990), in which similar subconvulsive soman exposure markedly interfered with schedule-controlled operant-conditioning performance.

Anticonvulsant treatments. The combination treatment with diazepam, HI-6 and atropine, referred to as "TT," effectively blocked soman convulsions in a majority of treated animals. An absence of seizures in soman-injected animals was found in 74% of TT-treated animals. Of those animals exhibiting seizures (i.e., 36%), convulsions and epileptiform spiking were relatively brief, averaging 32 sec. Similarly, TT also provided some degree of protection from the effects of soman on rPS, although the effects were quite variable across brain regions. Moreover, TT was only mildly protective against long-term neuropathology produced by soman, and TT was found to have significant toxic and lethal effects (>11%) of its own.

A similar mixture, to which had been added our proprietary neuroprotective agent, GMM_2, was much less lethal (<3%), while still providing good protection against convulsions and seizures. In addition, Q+ was able to prevent or reverse the major increases in permeability and to markedly reduce the neuropathology induced by lethal doses of soman.

Both Q+ and TT significantly reduced rPS in most regions when given in the absence of soman. These data provide evidence that one or more of the agents in TT is able to alter cerebrovascular function, and may partially explain the protective actions of this treatment mixture. The fact that the addition of GMM_2 (i.e.Q+ treatment) provided more complete protection and had greater effects on rPS supports this suggestion. These findings also support our original hypothesis that some of the neuropathology which follows soman intoxication may be due to damage to blood-brain-barrier functions. *In general, these findings indicate that more effective protection against long-term soman neurotoxicity may be possible through modification of the basic TT formulation, and that additional treatment strategies, such as those described below, should be explored.*

Exploratory studies with NECA: We have shown that NECA has obvious and powerful protective actions against seizure produced by soman. This protective action can be produced by equimolar or lower concentrations of NECA, and has been extremely consistent. Available data support a role for adenosine in the modulation of neuronal activity (Williams, 1984; Dunwiddie, 1985; Phillis and Wu, 1983; Fredholm and Hedqvist, 1980). Adenosine and its receptors have wide-spread distribution in the nervous system, and receptor activation is associated with both presynaptic and postsynaptic inhibitory effects (Kuroda and McIlwain, 1977; Stone, 1981; Bender et al, 1981; Pull and McIlwain, 1974. A variety of seizure models indicate that stable adenosine analogs have potent anticonvulsant effects (Albertson et al. (1983); Rosen and Berman, 1985; Dragunow and Goddard, 1985; Maitre et al., 1974; Dunwiddie and Worth, 1982; Murray et al., 1985; Burley and Ferrendelli, 1984; Turski et al, 1985).

Only a limited number of animals have been tested, and only at a few doses of NECA and soman. Future work would be required to explore the potential importance and usefulness of these agents, and activation of the brain adenosine system in general, in blocking soman-induced seizures, reducing its lethality, and protecting the nervous system from its damaging effects.

Table 1. Time-dependent convulsant effects on regional cerebral blood flow (rCBF): Pentylenetetrazol (Subconvulsant dose = 25 mg/kg, $0.6ED_{50}$).

BRAIN REGION	Control	1hr	1 day	1 week
I. Forebrain + Corpora quadrigemina				
Olfactory Bulb	1.46 ± .04	1.72 ± .07 *	1.60 ± .06	1.66 ± .04 *
Olfactory Tubercle	1.51 ± .05	1.85 ± .06 **	1.66 ± .06	1.71 ± .05 **
Cortex, Occipital	1.48 ± .05	1.93 ± .07 **	1.61 ± .04	1.73 ± .06 **
Parietal	1.81 ± .05	2.20 ± .04 **	1.95 ± .04	2.02 ± .08
Frontal	1.54 ± .04	2.03 ± .07 **	1.67 ± .05	1.78 ± .05 **
Piriform cortex/Amygdala	1.38 ± .04	1.80 ± .09 **	1.55 ± .04	1.61 ± .03 **
Basal Ganglia	1.36 ± .04	1.74 ± .09 **	1.52 ± .04	1.59 ± .05 **
Hippocampus	1.20 ± .04	1.47 ± .06 *	1.25 ± .03	1.35 ± .03
Colliculus, inferior	1.69 ± .05	1.87 ± .06	1.72 ± .05	1.80 ± .06
superior	1.59 ± .06	1.78 ± .07	1.63 ± .04	1.66 ± .05
Hypothalamus	1.41 ± .04	1.57 ± .07	1.51 ± .04	1.52 ± .04
Septal Area	1.19 ± .03	1.54 ± .09 **	1.30 ± .02	1.43 ± .04
II. Midbrain + Hindbrain				
Midbrain	1.45 ± .04	1.65 ± .07 *	1.53 ± .04	1.57 ± .04
Pons & Medulla	1.29 ± .04	1.47 ± .03	1.35 ± .04	1.40 ± .03
Cerebellum	1.34 ± .04	1.61 ± .05 **	1.37 ± .03	1.45 ± .05
Average Change from Control		+23%	+6%	+12%
Number of Animals	15	6	7	7

rCBF is expressed in ml/min x gm^{-1}, mean ± SEM. Post-hoc multiple range tests: Comparison groups = Saline vs 3 times after convulsant treatment; Student-Neuman-Keul's procedure, *p<.05; Duncan's procedure, ** p<.01.

Table 2. Time-dependent convulsant effects on regional cerebral blood flow (rCBF): Pentylenetetrazol (Convulsant dose = 50 mg/kg, 1.2ED_{50}).

BRAIN REGION	Control	1hr	1 day	1 week
I. Forebrain + Corpora quadrigemina				
Olfactory Bulb	1.46 ± .04	1.45 ± .06	1.51 ± .14	1.37 ± .10
Olfactory Tubercle	1.51 ± .05	1.41 ± .05	1.48 ± .14	1.43 ± .08
Cortex, Occipital	1.48 ± .05	1.48 ± .09	1.47 ± .11	1.50 ± .08
Parietal	1.81 ± .05	1.83 ± .10	1.80 ± .17	1.76 ± .10
Frontal	1.54 ± .04	1.26 ± .09 *	1.54 ± .13	1.51 ± .08
Piriform cortex/Amygdala	1.38 ± .04	1.01 ± .05 **	1.42 ± .13	1.39 ± .06
Basal Ganglia	1.36 ± .04	1.27 ± .06	1.38 ± .13	1.59 ± .05
Hippocampus	1.20 ± .04	1.16 ± .07	1.20 ± .11	1.27 ± .08
Colliculus, inferior	1.69 ± .05	1.69 ± .08	1.68 ± .09	1.71 ± .08
superior	1.59 ± .06	1.55 ± .07	1.52 ± .11	1.62 ± .08
Hypothalamus	1.41 ± .04	1.34 ± .05	1.44 ± .11	1.24 ± .20
Septal Area	1.19 ± .03	1.19 ± .06	1.28 ± .10	1.19 ± .06
II. Midbrain + Hindbrain				
Midbrain	1.45 ± .04	1.41 ± .05	1.42 ± .11	1.42 ± .08
Pons & Medulla	1.29 ± .04	1.29 ± .05	1.31 ± .11	1.30 ± .08
Cerebellum	1.34 ± .04	1.30 ± .06	1.24 ± .05	1.36 ± .06
Average Change from Control		-4%	-1%	+1%
Number of Animals	15	13	7	7

rCBF is expressed in ml/min x gm^{-1}, mean ± SEM. Post-hoc multiple range tests: Comparison groups = Saline vs 3 times after convulsant treatment; Student-Neuman-Keul's procedure, *p<.05; Duncan's procedure, ** p<.01.

Table 3. Time-dependent convulsant effects on regional permeability-capillary surface area products (rPS): Pentylenetetrazol (Subconvulsant dose = 25 mg/kg, $0.6ED_{50}$).

BRAIN REGION	Control	1hr	1 day	1 week
I. Forebrain + Corpora quadrigemina				
Olfactory Bulb	14.39 ± .87	14.40 ± 1.01	20.24 ± 2.51	15.76 ± 1.56
Olfactory Tubercle	6.73 ± 1.08	9.74 ± 1.18	13.63 ± 1.24 **	12.85 ± 1.32 **
Cortex, Occipital	6.00 ± .59	7.79 ± .59	8.65 ± .98 **	8.88 ± .98 *
Parietal	5.41 ± .70	5.43 ± .36	6.62 ± .57	7.01 ± .76
Frontal	5.88 ± .59	7.34 ± .49	8.80 ± .56 **	8.12 ± .72 *
Piriform cortex/Amygdala	5.19 ± .31	6.38 ± .90	6.83 ± .43	6.50 ± .73 *
Basal Ganglia	4.70 ± .95	3.94 ± .51	5.23 ± .36	4.62 ± .37
Hippocampus	5.00 ± .57	4.80 ± .43	5.78 ± .53	5.64 ± .69
Colliculus, inferior	7.51 ± .39	9.74 ± .97	10.50 ± .98 *	10.52 ± 1.08 **
superior	5.32 ± .50	6.96 ± .74	6.82 ± .69	7.27 ± .79 **
Hypothalamus	6.73 ± 1.05	6.67 ± .53	8.08 ± .59	7.70 ± .70
Septal Area	6.33 ± 1.25	9.36 ± .81	7.02 ± 1.02	8.61 ± 1.03 *
II. Midbrain + Hindbrain				
Midbrain	5.36 ± .90	4.77 ± .60	6.49 ± .27	4.82 ± .76
Pons & Medulla	9.62 ± .85	11.59 ± .66	14.40 ± .80 **	11.92 ± 1.52
Cerebellum	7.31 ± .51	8.98 ± .66	11.37 ± .79 **	10.09 ± 1.24 *
Average Change from Control		+13%	+37%	+31%
Number of Animals	10	9	8	7

rPS is shown as ml/sec x gm^{-1} x 10^6, mean ± SEM. Post-hoc multiple range tests: Comparison groups = Saline vs 3 times after convulsant treatment; Student-Neuman-Keul's procedure, *p<.05; Duncan's procedure, ** p<.01.

Table 4. Time-dependent convulsant effects on regional permeability-capillary surface area products (rPS): Pentylenetetrazol (Convulsant dose = 50 mg/kg, 1.2ED_{50}).

BRAIN REGION	Control	1hr	1 day	1 week
I. Forebrain + Corpora quadrigemina				
Olfactory Bulb	14.39 ± .87	14.88 ± 1.12	16.80 ± 1.76	12.31 ± 1.10
Olfactory Tubercle	6.73 ± 1.08	9.36 ± 1.26	7.03 ± 1.85	6.80 ± .66
Cortex, Occipital	6.00 ± .59	7.13 ± .97	5.67 ± .93	5.29 ± .73
Parietal	5.41 ± .70	7.71 ± 1.54	3.79 ± .87	4.07 ± .59
Frontal	5.88 ± .59	7.34 ± .79	4.90 ± .94	5.35 ± .61
Piriform cortex/Amygdala	5.19 ± .31	6.25 ± .55	5.00 ± .88	3.64 ± .35
Basal Ganglia	4.70 ± .95	5.73 ± .61	5.48 ± .88	3.71 ± .92
Hippocampus	5.00 ± .57	5.68 ± .68	4.21 ± 1.08	3.51 ± .55
Colliculus, inferior	7.51 ± .39	7.57 ± .74	5.30 ± 1.80	5.50 ± .83
superior	5.32 ± .50	5.93 ± .44	5.73 ± .87	4.98 ± .23
Hypothalamus	6.73 ± 1.05	7.57 ± .89	5.06 ± 1.02	6.49 ± .98
Septal Area	6.33 ± 1.25	7.53 ± 1.17	7.43 ± 3.06	4.00 ± .49
II. Midbrain + Hindbrain				
Midbrain	5.36 ± .90	5.30 ± .87	3.53 ± .56	3.48 ± .57
Pons & Medulla	9.62 ± .85	9.98 ± .80	8.23 ± .91	7.87 ± .59
Cerebellum	7.31 ± .51	8.23 ± .43	8.18 ± 1.10	6.49 ± .62
Average Change from Control		+17%	-3%	-19%
Number of Animals	10	10	6	7

rPS is shown as ml/sec x gm^{-1} x 10^6, mean ± SEM. Post-hoc multiple range tests: Comparison groups = Saline vs 3 times after convulsant treatment; Student-Neuman-Keul's procedure, *p<.05; Duncan's procedure, ** p<.01.

Table 5. Time-dependent convulsant effects on regional cerebral vascular space (BVS): Pentylenetetrazol (Subconvulsant dose = 25 mg/kg, $0.6ED_{50}$).

BRAIN REGION	Control	1hr	1 day	1 week
I. Forebrain + Corpora quadrigemina				
Olfactory Bulb	2.18 ± .16	1.75 ± .10	1.66 ± .15	1.85 ± .09
Olfactory Tubercle	2.26 ± .17	1.76 ± .19	1.78 ± .13	2.19 ± .15
Cortex, Occipital	1.29 ± .07	1.24 ± .10	1.09 ± .04	1.25 ± .11
Parietal	1.10 ± .10	0.91 ± .08	0.71 ± .04 *	0.85 ± .08
Frontal	1.24 ± .14	0.98 ± .08	0.89 ± .08	0.94 ± .08
Piriform cortex/Amygdala	1.06 ± .07	0.97 ± .14	0.68 ± .06 *	0.92 ± .09
Basal Ganglia	0.94 ± .12	0.71 ± .06	0.63 ± .06	0.70 ± .07
Hippocampus	0.78 ± .07	0.81 ± .11	0.60 ± .05	0.68 ± .06
Colliculus, inferior	1.50 ± .12	1.45 ± .14	1.33 ± .09	1.44 ± .12
superior	0.89 ± .11	0.71 ± .07	0.72 ± .06	0.78 ± .08
Hypothalamus	1.26 ± .11	1.09 ± .07	0.90 ± .06	1.13 ± .08
Septal Area	0.87 ± .12	0.75 ± .08	0.57 ± .06	0.70 ± .10
II. Midbrain + Hindbrain				
Midbrain	1.02 ± .09	0.92 ± .08	0.82 ± .06	0.95 ± .08
Pons & Medulla	2.55 ± .19	2.55 ± .19	2.42 ± .14	2.54 ± .10
Cerebellum	2.07 ± .12	1.90 ± .14	1.80 ± .10	1.82 ± .15
Average Change from Control		-12%	-23%	-12%
Number of Animals	14	12	12	10

BVS is expressed in ml/100 g tissue, mean ± SEM. Post-hoc multiple range tests: Comparison groups = Saline vs 3 times after convulsant treatments; Student-Neuman-Keul's procedure, *p<.05; Duncan's procedure, ** p<.01.

Table 6. Time-dependent convulsant effects on regional cerebral vascular space (BVS): Pentylenetetrazol (Convulsant dose = 50 mg/kg, $1.2ED_{50}$).

BRAIN REGION	Control	1hr	1 day	1 week
I. Forebrain + Corpora quadrigemina				
Olfactory Bulb	2.18 ± .16	2.07 ± .14	2.56 ± .16	2.04 ± .19
Olfactory Tubercle	2.26 ± .17	1.97 ± .17	1.99 ± .20	1.99 ± .33
Cortex, Occipital	1.29 ± .07	1.22 ± .08	1.47 ± .10	1.36 ± .10
Parietal	1.10 ± .10	1.03 ± .07	1.11 ± .09	1.06 ± .08
Frontal	1.24 ± .14	1.09 ± .06	1.28 ± .14	1.14 ± .12
Piriform cortex/Amygdala	1.06 ± .07	0.95 ± .05	1.03 ± .08	0.90 ± .11
Basal Ganglia	0.94 ± .12	0.66 ± .03	0.85 ± .10	0.77 ± .14
Hippocampus	0.78 ± .07	0.70 ± .04	0.88 ± .08	0.82 ± .07
Colliculus, inferior	1.50 ± .12	1.28 ± .11	1.49 ± .13	1.54 ± .09
superior	0.89 ± .11	0.82 ± .06	0.92 ± .09	0.90 ± .10
Hypothalamus	1.26 ± .11	1.13 ± .26	1.27 ± .11	1.01 ± .17
Septal Area	0.87 ± .12	0.79 ± .09	0.87 ± .10	0.94 ± .26
II. Midbrain + Hindbrain				
Midbrain	1.02 ± .09	0.86 ± .04	1.03 ± .09	0.95 ± .09
Pons & Medulla	2.55 ± .19	1.98 ± .13	2.01 ± .16	2.13 ± .23
Cerebellum	2.07 ± .12	1.64 ± .09	1.93 ± .17	1.67 ± .14
Average Change from Control		-12%	0%	-7%
Number of Animals	14	17	14	9

BVS is expressed in ml/100 g tissue, mean ± SEM. Post-hoc multiple range tests: Comparison groups = Saline vs 3 times after convulsant treatments; Student-Neuman-Keul's procedure, *p<.05; Duncan's procedure, ** p<.01.

Table 7. Histopathological (Nissl stain) scores in individual brain regions after exposure to convulsants and/or pretreatments.

	Group No.											
Region	1	2	3	4	5	6	7	8	9	10	11	12
Medullary reticular	1.0	2.8	1.5	1.5	2.8	2.3	1.5	2.0	1.5	2.3	1.0	2.0
Cerebellar cortex	1.0	2.2	1.0	2.0	2.4	1.3	1.0	1.0	1.0	1.0	1.0	2.0
Deep cerebellar n.	1.0	2.8	2.0	2.0	2.3	2.5	2.0	2.0	2.0	2.0	1.3	4.0
Inferior colliculus	1.3	2.4	1.5	3.5	3.9	3.3	1.5	2.0	1.5	2.7	1.3	3.0
Midbrain reticular	1.3	2.8	2.0	3.5	3.4	3.0	2.0	2.0	1.0	2.3	1.7	3.0
Superior colliculus	1.0	2.8	2.5	3.5	3.8	3.0	2.5	2.0	1.5	2.7	1.7	2.0
Occipital cortex	1.3	2.6	1.5	2.0	2.9	2.5	1.5	2.0	1.0	1.7	2.0	1.0
Hippocampus	1.0	2.2	1.5	2.5	3.4	2.3	1.0	2.0	1.0	1.7	1.5	2.0
Hippocampus ventral	1.0	1.0	1.0	1.0	1.4	1.0	1.0	2.0	1.0	2.0	1.0	1.0
Parietal cortex	1.3	2.8	1.0	3.0	3.1	3.0	2.0	2.0	2.0	3.7	2.0	2.0
Hypothal. PVN	1.0	1.4	1.5	1.5	1.9	1.0	1.0	1.3	1.0	1.0	1.0	1.0
Septal nuclei	1.0	2.4	3.5	3.5	2.5	3.0	2.5	2.3	1.0	3.0	2.7	1.0
Caudate putamen	1.3	2.8	3.0	3.5	3.6	3.0	2.5	2.0	2.0	3.0	3.0	3.0
Amygdala	1.0	2.4	2.0	2.0	3.6	2.5	1.5	1.3	2.0	2.0	2.0	1.0
Frontal cortex	1.3	2.6	2.0	2.0	3.1	2.8	1.5	2.3	1.5	3.3	2.0	2.0
Olfactory tubercle	1.0	2.0	1.5	2.0	3.3	2.0	1.0	2.0	1.0	2.7	1.5	1.0
Olfactory bulb	1.0	1.6	1.5	2.0	3.0	1.5	1.0	1.0	1.0	1.0	1.5	NA
Average	1.11	2.33	1.79	2.44	2.97	2.35	1.59	1.90	1.35	2.31	1.66	1.94
Number of Animals	3	5	2	2	8	4	2	3	2	3	4	1

Groups: 1. Saline controls; 2. PTZ (25 mg/kg); 3. PTZ (50 mg/kg); 4. Soman (33 µg/kg); 5. Soman (70 µg/kg); 6. TT only; 7. Quad+ only; 8. PTZ (50 mg/kg) + TT; 9. PTZ (50 mg/kg) + Quad+; 10. Soman(90 µg/kg) + TT; 11. Soman(90 µg/kg) + Quad+; 12. Soman(130 µg/kg) + NECA.

Injury scale: 1 = no cell damage; 2 = limited cell damage (5-10%), 3 = moderate cell damage (15-40%), 4 = severe cell damage (>50%).

Table 8. Time-dependent convulsant effects on regional cerebral blood flow (rCBF): Soman (Subconvulsant dose = 33 μg/kg, .50 LD_{50}).

BRAIN REGION	Control	1hr	1 day	1 week
I. Forebrain + Corpora quadrigemina				
Olfactory Bulb	1.46 ± .04	1.81 ± .13 **	1.50 ± .08	1.64 ± .04
Olfactory Tubercle	1.51 ± .05	1.89 ± .13 **	1.58 ± .08	1.63 ± .07
Cortex, Occipital	1.48 ± .05	2.11 ± .11 **	1.62 ± .07	1.59 ± .08
Parietal	1.81 ± .05	2.33 ± .12 **	1.90 ± .07	1.92 ± .10
Frontal	1.54 ± .04	2.21 ± .14 **	1.60 ± .08	1.71 ± .08
Piriform cortex/Amygdala	1.38 ± .04	1.81 ± .13 **	1.46 ± .07	1.53 ± .06
Basal Ganglia	1.36 ± .04	1.90 ± .15 **	1.51 ± .07	1.47 ± .07
Hippocampus	1.20 ± .04	1.67 ± .11 **	1.26 ± .08	1.25 ± .06
Colliculus, inferior	1.69 ± .05	1.92 ± .12 *	1.60 ± .08	1.71 ± .07
superior	1.59 ± .06	1.87 ± .14 *	1.53 ± .07	1.56 ± .07
Hypothalamus	1.41 ± .04	1.74 ± .17 *	1.43 ± .06	1.45 ± .07
Septal Area	1.19 ± .03	1.63 ± .15 **	1.26 ± .06	1.36 ± .06
II. Midbrain + Hindbrain				
Midbrain	1.45 ± .04	1.81 ± .15 **	1.45 ± .07	1.49 ± .08
Pons & Medulla	1.29 ± .04	1.52 ± .09 *	1.31 ± .07	1.32 ± .06
Cerebellum	1.34 ± .04	1.60 ± .11 *	1.34 ± .10	1.37 ± .05
Average Change from Control		+35%	+1%	+4%
Number of Animals	15	9	7	9

rCBF is expressed in ml/min x gm^{-1}, mean ± SEM. Post-hoc multiple range tests: Comparison groups = Saline vs 3 times after convulsant treatment; Student-Neuman-Keul's procedure, *p<.05; Duncan's procedure, ** p<.01.

Table 9. Time-dependent convulsant effects on regional cerebral blood flow (rCBF): Soman (Convulsant dose = 70 μg/kg, .93 LD_{50}).

BRAIN REGION	Control	1hr	1 day	1 week
I. Forebrain + Corpora quadrigemina				
Olfactory Bulb	1.46 ± .04	2.22 ± .08 **	1.65 ± .08	1.66 ± .06
Olfactory Tubercle	1.51 ± .05	2.30 ± .08 *	1.66 ± .07	1.70 ± .06
Cortex, Occipital	1.48 ± .05	2.40 ± .08 **	1.79 ± .10 *	1.73 ± .07
Parietal	1.81 ± .05	2.59 ± .09 **	2.26 ± .10 **	2.03 ± .08
Frontal	1.54 ± .04	2.48 ± .09 **	1.82 ± .12	1.74 ± .07
Piriform cortex/Amygdala	1.38 ± .04	2.37 ± .08 **	1.60 ± .08	1.57 ± .05
Basal Ganglia	1.36 ± .04	2.53 ± .11 **	1.65 ± .10 *	1.63 ± .06 *
Hippocampus	1.20 ± .04	2.16 ± .08 **	1.65 ± .11 **	1.39 ± .06
Colliculus, inferior	1.69 ± .05	2.26 ± .09 **	1.74 ± .06	1.84 ± .08
superior	1.59 ± .06	2.35 ± .09 **	1.59 ± .06	1.73 ± .07
Hypothalamus	1.41 ± .04	2.44 ± .09 **	1.74 ± .08 **	1.61 ± .06
Septal Area	1.19 ± .03	2.37 ± .09 **	1.86 ± .10 **	1.49 ± .07 **
II. Midbrain + Hindbrain				
Midbrain	1.45 ± .04	2.40 ± .10 **	1.68 ± .07 *	1.64 ± .08
Pons & Medulla	1.29 ± .04	1.77 ± .10 **	1.26 ± .05	1.44 ± .06
Cerebellum	1.34 ± .04	1.88 ± .11 **	1.21 ± .04	1.51 ± .06
Average Change from Control		+69%	+16%	+12%
Number of Animals	15	9	10	9

rCBF is expressed in ml/min x gm^{-1}, mean ± SEM. Post-hoc multiple range tests: Comparison groups = Saline vs 3 times after convulsant treatment; Student-Neuman-Keul's procedure, *p<.05; Duncan's procedure, ** p<.01.

Table 10. Time-dependent convulsant effects on regional permeability-capillary surface area products (rPS): Soman (Subconvulsant dose = 33 μg/kg, .50 LD_{50}).

BRAIN REGION	Control	1hr	1 day	1 week
I. Forebrain + Corpora quadrigemina				
Olfactory Bulb	14.39 ± .87	18.98 ± 1.61	18.06 ± .97	19.63 ± 1.35 **
Olfactory Tubercle	6.73 ± 1.08	12.91 ± 2.05	10.76 ± 1.41	11.61 ± 1.41 **
Cortex, Occipital	6.00 ± .59	8.51 ± .99	6.33 ± .27	9.33 ± 1.06
Parietal	5.41 ± .70	6.54 ± 1.50	6.31 ± .63	6.41 ± .81
Frontal	5.88 ± .59	6.83 ± 1.06	6.79 ± .31	8.44 ± .80
Piriform cortex/Amygdala	5.19 ± .31	5.68 ± .38	4.97 ± .68	6.10 ± .76
Basal Ganglia	4.70 ± .95	2.96 ± .54	3.40 ± .35	4.55 ± .85
Hippocampus	5.00 ± .57	4.66 ± .56	4.70 ± .79	5.09 ± .86
Colliculus, inferior	7.51 ± .39	7.26 ± 1.00	7.71 ± .80	9.64 ± 1.43
superior	5.32 ± .50	4.02 ± .67	3.70 ± .34	6.22 ± .94
Hypothalamus	6.73 ± 1.05	6.48 ± 1.13	7.05 ± .74	8.20 ± .97
Septal Area	6.33 ± 1.25	3.46 ± 1.08	4.67 ± .53	5.72 ± .65
II. Midbrain + Hindbrain				
Midbrain	5.36 ± .90	5.00 ± .49	5.12 ± .23	6.13 ± .82
Pons & Medulla	9.62 ± .85	14.81 ± 1.26 **	16.77 ± 1.38 **	15.23 ± .99 **
Cerebellum	7.31 ± .51	13.91 ± 1.42 **	12.99 ± .34 **	11.80 ± .89 **
Average Change from Control		+10%	+18%	+31%
Number of Animals	10	6	7	8

rPS is shown as ml/sec x gm^{-1} x 10^{6}, mean ± SEM. Post-hoc multiple range tests: Comparison groups = Saline vs 3 times after convulsant treatment; Student-Neuman-Keul's procedure, *p<.05; Duncan's procedure, ** p<.01.

Table 11. Time-dependent convulsant effects on regional permeability-capillary surface area products (rPS): Soman (Convulsant dose = 70 μg/kg, .93 LD_{50}).

BRAIN REGION	Control	1hr	1 day	1 week
I. Forebrain + Corpora quadrigemina				
Olfactory Bulb	14.39 ± .87	29.39 ± 1.90 **	16.13 ± 1.58	18.64 ± 1.50 **
Olfactory Tubercle	6.73 ± 1.08	20.68 ± 1.80 **	16.22 ± 3.78 **	12.97 ± 1.08 **
Cortex, Occipital	6.00 ± .59	8.51 ± .99	17.32 ± 1.49 **	8.03 ± .83
Parietal	5.41 ± .70	12.65 ± 1.14 **	10.04 ± 1.25 **	5.91 ± .98
Frontal	5.88 ± .59	13.58 ± 1.06 **	16.35 ± 2.30 **	7.64 ± .77
Piriform cortex/Amygdala	5.19 ± .31	13.52 ± 1.11 **	11.53 ± 1.08 **	5.95 ± .64
Basal Ganglia	4.70 ± .95	15.75 ± 2.05 **	4.35 ± .60	3.58 ± .72
Hippocampus	5.00 ± .57	13.40 ± 1.52 **	7.47 ± .75 *	5.00 ± .59
Colliculus, inferior	7.51 ± .39	12.54 ± 1.73 **	7.44 ± .98	7.22 ± .95
superior	5.32 ± .50	9.18 ± 1.09 **	3.04 ± .58 *	3.12 ± .71
Hypothalamus	6.73 ± 1.05	19.14 ± 2.21 **	9.61 ± 1.10 **	7.56 ± .72
Septal Area	6.33 ± 1.25	17.67 ± 2.15 **	4.40 ± .61	2.72 ± 1.07
II. Midbrain + Hindbrain				
Midbrain	5.36 ± .90	16.75 ± 1.91 **	8.91 ± .90 **	5.70 ± .64
Pons & Medulla	9.62 ± .85	22.89 ± 1.40 **	17.41 ± 1.70 **	17.18 ± 1.47 **
Cerebellum	7.31 ± .51	17.94 ± .99 **	12.35 ± .96 **	12.47 ± 1.20 **
Average Change from Control		+142%	+64%	+17%
Number of Animals	10	10	7	9

rPS is shown as ml/sec x gm^{-1} x 10^{6}, mean ± SEM. Post-hoc multiple range tests: Comparison groups = Saline vs 3 times after convulsant treatment; Student-Neuman-Keul's procedure, *p<.05; Duncan's procedure, ** p<.01.

Table 12. Time-dependent convulsant effects on regional cerebral vascular space (BVS): Soman (Subconvulsant dose = 33 μg/kg, .50 LD_{50}).

BRAIN REGION	Control	1hr	1 day	1 week
I. Forebrain + Corpora quadrigemina				
Olfactory Bulb	2.18 ± .16	1.48 ± .12 **	1.52 ± .17 *	1.78 ± .13
Olfactory Tubercle	2.26 ± .17	1.87 ± .23	1.80 ± .27	2.26 ± .19
Cortex, Occipital	1.29 ± .07	1.22 ± .09	1.03 ± .12	1.33 ± .08
Parietal	1.10 ± .10	0.78 ± .12	0.69 ± .07 *	0.82 ± .10
Frontal	1.24 ± .14	0.85 ± .10	0.83 ± .10	1.04 ± .13
Piriform cortex/Amygdala	1.06 ± .07	0.73 ± .08	0.72 ± .10 *	0.81 ± .06 **
Basal Ganglia	0.94 ± .12	0.59 ± .11	0.61 ± .09	0.68 ± .08
Hippocampus	0.78 ± .07	0.73 ± .10	0.72 ± .09	0.75 ± .07
Colliculus, inferior	1.50 ± .12	1.35 ± .18	1.17 ± .12	1.62 ± .14
superior	0.89 ± .11	0.85 ± .15	0.69 ± .10	0.95 ± .10
Hypothalamus	1.26 ± .11	0.89 ± .16	0.99 ± .13	1.11 ± .09
Septal Area	0.87 ± .12	0.74 ± .14	0.79 ± .09	1.01 ± .12
II. Midbrain + Hindbrain				
Midbrain	1.02 ± .09	0.75 ± .09	0.76 ± .08	0.91 ± .08
Pons & Medulla	2.55 ± .19	1.91 ± .19	2.28 ± .17	2.51 ± .24
Cerebellum	2.07 ± .12	1.47 ± .13 *	1.49 ± .11 *	1.84 ± .33
Average Change from Control		-22%	-24%	-8%
Number of Animals	14	7	7	10

BVS is expressed in ml/100 g tissue, mean ± SEM. Post-hoc multiple range tests: Comparison groups = Saline vs 3 times after convulsant treatments; Student-Neuman-Keul's procedure, *p<.05; Duncan's procedure, ** p<.01.

Table 13. Time-dependent convulsant effects on regional cerebral vascular space (BVS): Soman (Convulsant dose = 70 μg/kg, .93 LD_{50}).

BRAIN REGION	Control	1hr	1 day	1 week
I. Forebrain + Corpora quadrigemina				
Olfactory Bulb	2.18 ± .16	2.13 ± .09	1.47 ± .14 **	1.81 ± .18
Olfactory Tubercle	2.26 ± .17	2.34 ± .10	2.04 ± .19	2.13 ± .14
Cortex, Occipital	1.29 ± .07	1.68 ± .17	1.65 ± .12 *	1.25 ± .07
Parietal	1.10 ± .10	1.13 ± .13	0.98 ± .09	0.82 ± .07 *
Frontal	1.24 ± .14	1.30 ± .14	1.30 ± .11	1.08 ± .08
Piriform cortex/Amygdala	1.06 ± .07	1.16 ± .13	1.03 ± .08	0.92 ± .08 *
Basal Ganglia	0.94 ± .12	0.87 ± .10	0.58 ± .05	0.78 ± .07
Hippocampus	0.78 ± .07	1.06 ± .12 *	0.77 ± .06	0.74 ± .06
Colliculus, inferior	1.50 ± .12	1.92 ± .24	1.22 ± .09	1.37 ± .09
superior	0.89 ± .11	1.18 ± .13 **	0.66 ± .07	0.75 ± .04
Hypothalamus	1.26 ± .11	1.39 ± .15	1.01 ± .06	1.19 ± .07
Septal Area	0.87 ± .12	1.32 ± .15 *	1.01 ± .07	0.88 ± .05
II. Midbrain + Hindbrain				
Midbrain	1.02 ± .09	1.21 ± .14	0.91 ± .20	0.88 ± .06
Pons & Medulla	2.55 ± .19	2.65 ± .20	2.07 ± .17	2.54 ± .13
Cerebellum	2.07 ± .12	1.96 ± .15	1.33 ± .12 **	1.60 ± .13
Average Change from Control		+15%	-12%	-11%
Number of Animals	14	10	9	10

BVS is expressed in ml/100 g tissue, mean ± SEM. Post-hoc multiple range tests: Comparison groups = Saline vs 3 times after convulsant treatments; Student-Neuman-Keul's procedure, *p<.05; Duncan's procedure, ** p<.01.

Table 14. Effects on regional permeability-capillary surface area products (rPS) measured 24 hr after treatments. Comparisons of three non-convulsant control groups: Saline vs "triple treat-TT" or "quad plus-Q+".

BRAIN REGION	Saline	TT	Q+
I. Forebrain + Corpora quadrigemina			
Olfactory Bulb	14.39 ± .87	15.63 ± 1.44	14.53 ± .75
Olfactory Tubercle	6.73 ± 1.08	7.73 ± .58	6.15 ± .56
Cortex, Occipital	6.00 ± .59	5.73 ± .58	4.80 ± .43
Parietal	5.41 ± .70	4.39 ± .40	3.70 ± .22
Frontal	5.88 ± .59	5.94 ± .43	4.69 ± .38
Piriform cortex/Amygdala	5.19 ± .31	4.15 ± .43 *	4.24 ± .32 *
Basal Ganglia	4.70 ± .95	1.79 ± .30 **	2.33 ± .28 *
Hippocampus	5.00 ± .57	3.14 ± .46 **	2.31 ± .19 **
Colliculus, inferior	7.51 ± .39	5.84 ± .68 *	4.26 ± .53 **
superior	5.32 ± .50	2.81 ± .51 **	2.43 ± .52 **
Hypothalamus	6.73 ± 1.05	5.74 ± .72	4.64 ± .23
Septal Area	6.33 ± 1.25	3.61 ± .51 *	2.81 ± .41 **
II. Midbrain + Hindbrain			
Midbrain	5.36 ± .90	3.96 ± .36	3.69 ± .31
Pons & Medulla	9.62 ± .85	13.47 ± 1.30	12.19 ± .99
Cerebellum	7.31 ± .51	9.24 ± .87	9.68 ± .72
Average Change from Saline Control		-14%	-23%
Number of Animals	10	8	9

rPS is shown as ml/sec x gm^{-1} x 10^6, mean ± SEM. Post-hoc multiple range tests:
Comparisons = 2 treatments; Student-Neuman-Keul's procedure, *p<.05; Duncan's procedure, ** p<.01.

TT = triple SC pre-treatment with atropine (15mg/kg), HI6 (125 mg/kg), diazepam (2 mg/kg).
Q+ = triple SC pre-treatment with atropine, HI6, and diazepam plus GMM_2 (70 µg/kg followed 5-6 hr later with another single SC injection of GMM_2, 70 µg/kg.

Table 15. Effects on regional permeability-capillary surface area products (rPS) measured 1 day after various protective treatments compared in normal or unprotected convulsed animals.

Pentylenetetrazol (55 μg/kg, 1.3ED_{50})

BRAIN REGION	Saline, no convulsant	Triple treat	Convulsant plus Quad+	Saline only
I. Forebrain + Corpora quadrigemina				
Olfactory Bulb	14.39 ± .87	13.18 ± 1.14	17.83 ± 1.56	16.80 ± 1.76
Olfactory Tubercle	6.73 ± 1.08	7.86 ± .88	7.56 ± .51	7.02 ± 1.85
Cortex, Occipital	6.00 ± .59	4.71 ± .36	5.31 ± .19	5.67 ± .93
Parietal	5.41 ± .70	3.25 ± .23 **	4.55 ± .16	3.79 ± .87
Frontal	5.88 ± .59	4.65 ± .50	5.04 ± .21	4.90 ± .94
Piriform cortex/Amygdala	5.19 ± .31	4.00 ± .51	4.18 ± .27	5.00 ± .88
Basal Ganglia	4.70 ± .95	1.46 ± .13 **	2.53 ± .12 *	5.48 ± .88
Hippocampus	5.00 ± .57	2.23 ± .20 **	3.13 ± .13 **	4.21 ± 1.08
Colliculus, inferior	7.51 ± .39	4.49 ± .45 **	5.45 ± .27 *	5.30 ± 1.80
superior	5.32 ± .50	1.98 ± .19 **	3.43 ± .21 **	5.73 ± .87
Hypothalamus	6.73 ± 1.05	4.81 ± .28	4.97 ± .21	5.06 ± 1.02
Septal Area	6.33 ± 1.25	2.24 ± .22 **	3.92 ± .31	7.43 ± 3.06
II. Midbrain + Hindbrain				
Midbrain	5.36 ± .90	3.17 ± .18 *	3.81 ± .26	3.53 ± .56
Pons & Medulla	9.62 ± .85	12.50 ± .49 *	12.26 ± .83 *	8.23 ± .91
Cerebellum	7.31 ± .51	8.91 ± .54	9.13 ± .49	8.18 ± 1.10
Average Change from Control		-27%	-14%	-7%
Number of Animals	10	11	7	6

rPS is shown as ml/sec x gm^{-1} x 10^6, mean ± SEM. Post-hoc multiple range tests: Comparisons groups = saline vs 3 treated convulsion groups; Student-Neuman-Keul's procedure, *p<.05; Duncan's procedure, ** p<.01.

Table 16. Effects on regional permeability-capillary surface area products measured 1 day after various protective treatments compared in normal or unprotected convulsed animals.

BRAIN REGION	Saline, no convulsant	Soman (90 μg/kg, 1.3ED_{50}, 1.2LD_{50}) Triple treat	Convulsant plus Quad+	Saline only
I. Forebrain + Corpora quadrigemina				
Olfactory Bulb	14.39 ± .87	18.04 ± 1.71	11.62 ± .85	16.13 ± 1.58
Olfactory Tubercle	6.73 ± 1.08	10.54 ± 1.74	3.61 ± .44	16.22 ± 1.74 **
Cortex, Occipital	6.00 ± .59	8.16 ± .74 *	2.85 ± .22 **	17.33 ± 1.49 **
Parietal	5.41 ± .70	7.74 ± .62 *	2.48 ± .17 **	10.04 ± 1.25 **
Frontal	5.88 ± .59	7.63 ± 1.07	2.44 ± .27 *	16.35 ± 2.30 **
Piriform cortex/Amygdala	5.19 ± .31	7.89 ± .79 **	2.73 ± .32 **	11.53 ± 1.08 **
Basal Ganglia	4.70 ± .95	8.11 ± 1.36 **	1.17 ± .12 **	4.35 ± .60
Hippocampus	5.00 ± .57	7.54 ± 1.04 *	2.14 ± .23 **	7.47 ± .75 *
Colliculus, inferior	7.51 ± .39	8.77 ± 1.01	3.60 ± .41 *	7.44 ± .98
superior	5.32 ± .50	6.32 ± .82	1.77 ± .23 **	3.04 ± .58 **
Hypothalamus	6.73 ± 1.05	9.88 ± 1.15 **	3.41 ± .31 **	9.61 ± 1.10 *
Septal Area	6.33 ± 1.25	2.87 ± .60 **	1.12 ± .32 **	4.40 ± .61
II. Midbrain + Hindbrain				
Midbrain	5.36 ± .90	7.56 ± .92 *	3.27 ± .27 *	8.91 ± .90 **
Pons & Medulla	9.62 ± .85	15.25 ± 1.73 **	13.03 ± .75 *	17.41 ± 1.70 **
Cerebellum	7.31 ± .51	10.21 ± 1.15 *	9.90 ± .59 *	12.35 ± .96 **
Average Change from Control		+36%	-42%	+64%
Number of Animals	10	8	12	7

rPS is shown as ml/sec x gm^{-1} x 10^6, mean ± SEM. Post-hoc multiple range tests: Comparisons groups = saline vs 3 treated convulsion groups; Student-Neuman-Keul's procedure, *p<.05; Duncan's procedure, ** p<.01.

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